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Appendix

New-Generation Steroid Hormones

Tailored Assessment Strategies for Environmental Protection

by:

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List of abbreviations

Anova	Analysis of Variance
(d)pf	(days) post fertilisation
ELISA	Enzyme linked immunosorbent assay
ELS	Early life stage
F_0 generation	Parental generation (F_0)
F_1 generation	First filial generation (F_1)
F_2 generation	Second filial generation (F_2)
FLCT	Fish life cycle test
FSDT	Fish sexual development test
FSTRA	Fish short term reproduction assay
IME	(Fraunhofer) Institute for Molecular Biology and Applied Ecology
LC MS	Liquid Chromatography - Mass Spectrometry
LOEC	Lowest observed effect concentration
LPS	Lipopolysaccharides
MEOGRT	Medaka extended one generation reproduction test
MoA	Mode of action
NOEC	No observed effect concentration
OECD	Organisation for Economic Co-operation and Development
PaMP	Pathogen-associated molecular pattern
pf	Post fertilisation
RSD	Relative standard deviation
SD	Standard deviation
SOP	Standard operation procedure
TIU	Trypsin Inhibitor Unit
UBA	Umweltbundesamt (German Environment Agency)

B Appendix

B.1 Analytical report for Dienogest – Details of the method and results

B.1.1 Scope

The purpose of this analytical part of the study was to determine the analyte Dienogest in aqueous test medium (holding and dilution water) and sediment. In total three ecotoxicological studies were conducted. Aqueous samples were taken and measured from a Zebrafish extended one generation reproduction test (ZEOGRT) and a Chironomid life cycle test. The Chironomid study furthermore required the analysis of sediment samples. As an additional study, a *Daphnia magna* reproduction test (according to OECD TG 211) was conducted at the laboratory of the German Federal Agency (UBA). Samples from that study were shipped to Fraunhofer IME and measured. Results of that measurements are presented in this Appendix. The quantitative measurements were done by liquid chromatography coupled to a triple quadruple mass spectrometer; the MS was operated in the tandem mass spectrometry mode (LC-MS/MS).

The active substance Dienogest (CAS no. 65928-58-7) was also used as the analytical standard; isotopically labelled Dienogest-d6 was used as internal standard (IS).

B.1.2 Chemicals, reagents and analytical equipment

- Dienogest, active substance, Batch: S125103, Purity (HPLC): 99.88%, Stability: 3 years at -20°C, Article/catalog no.: S1251 (Selleckchem.com), see CoA in B.1.8
- Internal Standard, Dienogest-d6, Lot: 055, Enrichment: 99%, Expiry Date: not specified, Article/product: D75937 (Medical Isotopes Inc.), see data sheet in B.1.9
- IS solution, solution of the internal standard Dienogest-d6 in methanol, conc.: 10 µg/L
- Acetone, 'Acetone for residue analysis', Article No. 2661 (Th. Geyer)
- Methanol, 'Methanol for LC-MS', Article No. 1428 (Th. Geyer)
- Acetonitrile, 'Acetonitrile for LC-MS', Article No. 2697 (Th. Geyer)
- Ammonium acetate, 'Optima LC-MS', Article No. 11317490 (Fisher Scientific)
- Purified water, produced with purification system Purelab® Ultra (ELGA LabWater)
- Solvent mixture 1: Mixture of 1 part methanol and 10 parts dilution water
- Analytical balance XPE 205 DR (Mettler Toledo)
- Piston operated pipette, 'research 5000', variable volume selection (Eppendorf)
- Glass funnel
- 12 mL screw cap vials, 15 mm thread, 66 x 19 mm, clear glass; equipped with 15 mm screw caps with Teflon coated sealing disks (WiCom)
- 40 mL screw cap vials, 95 x 27.5 mm, clear glass; equipped with screw caps with Teflon coated sealing disks (VWR)

- 70 mL centrifuge glas, 100 x 44 mm, clear glass; equipped with screw caps with Teflon coated sealing disks
- 100 mL volumetric flask
- 25 mL volumetric flask
- Common laboratory equipment (volumetric flasks, glass beakers, Pasteur pipettes etc.)
- Screw top vials, approx. 2.0 mL capacity, clear glass; screw caps with Teflon coated sealing disks (WiCom)
- MicromanTM pipettes (positive displacement), M25, M50 and M250 (Fisher Scientific)

B.1.3 Sampling and sample processing

Sampling

Sampling of aqueous medium was performed by the staff of the ecotoxicology department using piston operated pipettes. To prevent degradation of the analyte and to minimize wall effects the aqueous samples were stabilized by addition of methanol.

Chironomid Life cycle test: Aliquots of precisely 10 mL methanol were filled into each 40 mL screw cap vial and mixed with exactly 10 mL of the aqueous test media samples. 20 µL were transferred to a 12 mL glass vial and diluted with 9.98 mL of the solvent mixture (water+methanol 10+1; dilution 1:1000 based on the initial aqueous part). The samples were measured directly by LC-MS/MS.

Sampling of sediment samples was also performed by the staff of the ecotoxicology department weighing ca. 12-14 g wet weight (corresponds to ca. 10 g of dry weight) of the sediment into 70 mL centrifuge glass vials. 33 mL of acetone were added, the vials closed with the screw caps and the samples shaken at a setting of 200 rpm on a horizontal shaker for 20 min. Then samples were centrifuged at 4000 rpm for 5 minutes. The extract was transferred to a 100 mL volumetric flask using a glass funnel. This extraction was repeated twice with the same amount of acetone. Finally, the volumetric flask was made up to the ring mark with acetone and gently shaken to homogenize the extracts.

Zebrafish EOGRT: Aliquots of accurately 0.50 mL methanol were filled into each 12 mL screw cap vials prior to the start of the sampling procedure. Subsequent 5.00 mL of the aqueous test media were taken out of the test vessels and were pipetted into the screw top vials as well; the volume mix water/methanol was therefore 10+1 (v/v). After vigorously mixing by hand the samples were stored in a freezer at a temperature of ≤ -18°C until analysis.

Daphnia magna reproduction test: 42 samples originated from an external study. 5 mL of test medium had been stabilized with 0.5 mL of methanol. Samples were kept frozen until further processing and analysis.

Sample processing

For direct LC-MS/MS analysis of the prepared aqueous samples these were allowed to equilibrate at room temperature.

Chironomid Life Cycle Test: 20 µL were transferred to a 12 mL glass vial and diluted with 9.98 mL of the solvent mixture (dilution 1:1000). 1000 µL of this solution were mixed with 25 µL of the internal standard solution (10 µg/L Dienogest-d6 in methanol) before analysis by LC-MS/MS. For analysis of the sediment samples 25 µL of the acetone extract were transferred to a 25 mL volumetric flask and made up to the ringmark with methanol (dilution 1:1000). 100 µL were transferred to a HPLC vial, 1000 µL of copper reduced water and 25 µL of the internal standard solution (10 µg/L Dienogest-d6 in methanol) were added before analysis by LC-MS/MS.

Zebrafish EOGRT: For direct LC-MS/MS measurement the samples were thawed and allowed to equilibrate to room temperature. Aliquots of 1.10 mL of the aqueous samples (water/methanol mixtures) were then filled into 2 mL screw top vials; afterwards 25.0 µL of the IS solution IS-IM-2a were added and the vials were mixed by hand. Finally, 50 µL of the processed sample were injected into the LC-MS/MS system, the analytical method is described in detail in chapter B.1.4. The remaining water/methanol mixtures were stored in a freezer at a temperature of ≤ -18 °C to allow a second measurement.

Daphnia magna reproduction test: Samples were thawed and allowed to equilibrate at room temperature. 4.5 mL of methanol were added (dilution 1+1 v/v methanol/testmedium). 20 µL of this dilution were transferred to a 12 mL glass vial and diluted with 9.98 mL of the solvent mixture (dilution 1:500). Taking both dilution steps into consideration the final dilution equals 1:1000. Exactly 1 mL was mixed with 25 µL of the internal standard solution (10 µg/L Dienogest-d6 in methanol) before analysis by LC-MS/MS.

B.1.4 LC-MS/MS measurement

The quantitative determination of Dienogest was carried out by liquid chromatography and tandem mass spectrometry detection (LC-MS/MS) using positive electrospray ionization (ESI+). The measurement conditions and instrument settings are summarized below.

LC-MS/MS system

HPLC system:	Waters Acquity UPLC H-Class System
Mass spectrometer:	Waters LC-MS/MS Xevo TQ-S (triple quadruple system)
Software:	Waters MassLynx Ver. 4.1
Quantitation software:	Waters QuanLynx Ver. 4.1

LC parameters

Column:	Acquity UPLC BEH C18; 1.7 µm, 50 mm x 2.1 mm
Column temperature:	40°C
Injection volume:	50 µL
Flow rate:	0.30 mL/min
Mobile phase A:	1000 mL acetonitrile + 2mL 1 M ammonium acetate solution
Mobile phase B:	950 mL purified water + 50 mL acetonitrile + 2 mL 1 M ammonium acetate solution

Gradient program

Time [min]	Solvent A [%]	Solvent B [%]
0.00	10	90
0.20	20	90
4.00	50	50
4.10	100	0
6.60	100	0
6.61	10	90
8.00	10	90

MS method (measurement conditions and instrument setting)

Type: MRM
Ion mode: ESI+
Span: 0.2 Da
Solvent delay 1: 0.10 – 3.90 min
Solvent delay 2: 4.90 – 7.95 min
End time: 8.00 min
Collision gas: Argon

Usage of MRM transition

Substance (indication)	Retention time [min]	Precursor ion [m/z]	Product ion [m/z]	Cone voltage [V]	Collision energy [eV]	Dwell time [s]
Analyte, quantitation ion	4.25	312.28	160.98	30	25	0.100
Analyte, qualifier ion		312.28	135.02	30	28	0.100
IS, quantitation ion	4.25	318.34	139.03	30	30	0.100
IS, qualifier ion		318.34	167.03	30	27	0.100

MS parameters

Source settings		Analyzer settings	
Capillary:	0.80 kV	LM 1 resolution:	2.9
Source Offset:	50.0 V	HM 1 resolution:	15.0
Source temperature:	150°C	Ion energy 1:	0.7
Desolvation temperature:	600°C	MS Mode Entrance:	1
Cone gas flow:	150 L/h	MS Mode Exit:	1
Desolvation gas flow:	1000 L/h	LM 2 resolution:	2.7
Collision gas flow:	0.18 L/min	HM 2 resolution:	15.0
Nebulizer gas flow:	7.00 bar	Ion energy 2:	2.0
		Gain (multiplier)	1.00

B.1.5 Calibration, quantification and calculation of the analytical result

Stock and intermediate solutions of the analyte and the internal standard

The stock solutions of active substance (analyte) Dienogest and the internal standard (IS) Dienogest-d_6 were prepared by exact weighing the standard compounds directly into separate volumetric flasks and by subsequent filling up to the ring mark with methanol, see Table 1.

Table 1: Preparation of stock solutions of the analyte and internal standard.

Stock solution	Compound	Date of preparation	Weighed amount	Purity	Volumetric flask, nominal volume	Resulting concentration
S-1a	Dienogest	Sept. 03, 2018	20.35 mg	99.88%	20 mL	1.016 g/L
S-1b	Dienogest	Sept. 07, 2018	20.17 mg	99.88%	20 mL	1.007 g/L
S-1c	Dienogest	Nov. 29, 2018	20.83 mg	99.88%	20 mL	1.040 g/L
IS-1a	Dienogest-d6	Sept. 06, 2018	1.01 mg*	99%	5 mL	0.200 g/L

* Total quantity delivered by supplier.

For the preparation of the calibration solutions intermediate solutions (IM) were prepared by pipetting aliquots of the analyte stock S-1a or the IM solution IM-1a into additional volumetric flasks and filling up to the mark with methanol, the resulting concentrations and the dilution scheme are given in Table 2.

Table 2: Preparation of analyte intermediate solution (MI set 1).

Analyte intermediate (IM) solution	Date of preparation	Used solution	Used volume	Volumetric flask, nominal volume	Resulting concentration
IM-1a	Sept. 03, 2018	S-1a	49.2 µL	25	2.00 mg/L
IM-2a	Sept. 03, 2018	IM-1a	50.00 µL	20	5.00 µg/L
IM-1c	Nov.29, 2018	S-1c	48.07 µL	25 mL	2.00 mg/L
IM-2c	Nov.29, 2018	IM-1c	50.00 µL	20 mL	5.00 µg/L

For spiking the test media samples during sample processing and preparation of the calibration solutions an IM solution of the IS was prepared by dilution with methanol, the resulting concentration and the dilution scheme is given in Table 3.

Table 3: Preparation of internal standard spiking solution.

IS intermediate (IM)solution	Date of preparation	Used solution	Used volume	Volumetric flask, nominal volume	Resulting concentration
IS-IM-1a	Sept. 06, 2018	IS-1a	50.00 µL	10 mL	1.00 mg/L
IS-IM-2a	Sept. 06, 2018	IS-IM-1a	250.0 µL	25 mL	10.0 µg/L

For the preparation of the QC standards an additional set of analyte IM solutions was prepared in methanol based on the analyte stock S-1b; the solutions, the dilution scheme and the resulting concentrations are given in the Table 4.

Table 4: Preparation of analyte intermediate solution (IM set 2).

Analyte intermediate (IM) solution	Date of preparation	Used solution	Used volume	Volumetric flask, nominal volume	Resulting concentration
IM-1b	Sept. 07, 2018	S-1b	49.64 µL	25 mL	2.00 mg/L
IM-2b	Sept. 03, 2018	IM-1b	50.00 µL	20 mL	5.00 µg/L

All stock and intermediate solutions were stored at a temperature of approx. 4°C in a refrigerator.

Preparation of the calibration standards and the matrix calibration samples

Due to the expected higher stability of the analyte in an organic solvent the 'calibration standards' (calibration solutions) were prepared and stored in pure methanol.

Nine calibration standards (C-1a, C-2a, …) were prepared on September 06, 2018 in the concentration range from 1.00 to 500 ng/L by diluting the intermediate solution IM-2a in volumetric flasks with methanol; see pipetting plan in Table 5 (microman pipettes were used for this dilution step).

The 'matrix calibration samples' (CS-1, CS-2, …) were prepared afterwards by mixing 100 µL of the calibration standards and 25 µL of the IS solution IS-IM-2a with 1000 µL aqueous test medium (copper reduced water for sediment samples) in 2 mL screw top vials; the volume mix water/methanol was therefore 10+1 (v/v), this was the same solvent composition as it existed at the end of sample processing, cp. chapter B.1.3. The calibration samples were measured as described in chapter B.1.4.

All prepared calibration standards were stored at approximately 4°C in a refrigerator.

Table 5: Preparation of the calibration standards and the calibration samples, analyte Dienogest.

No. of the calibration solution	Volume solution IM-2a (2c)	Volumetric flask nominal volume	Analyte concentration, calibration solution	No. of the calibration sample	Analyte concentration, calibration sample*
C-1c	20.00 µL	10 mL	10.0 ng/L	CS-1	1.00 ng/L
C-2c	50.00 µL	10 mL	25.0 ng/L	CS-2	2.50 ng/L
C-3c	100.0 µL	10 mL	50.0 ng/L	CS-3	5.00 ng/L
C-4c	250.0 µL	10 mL	125 ng/L	CS-4	12.5 ng/L
C-5c	500.0 µL	10 mL	250 ng/L	CS-5	25.0 ng/L
C-6c	1000 µL	10 mL	500 ng/L	CS-6	50.0 ng/L
C-7c	2000 µL	10 mL	1000 ng/L	CS-7	100 ng/L
C-8c	5000 µL	10 mL	2500 ng/L	CS-8	250 ng/L
C-9c	10000 µL	10 mL	5000 ng/L	CS-9	500 ng/L

*) Remark: The concentrations of the final calibration samples were related to the analyte amounts in the methanol/water mixture. They are lower than the actual concentrations as the added volumes of the calibration standards (CS-1, CS-2, …) and the IS solution are not considered. These concentrations correspond to the concentrations of test samples to be analyzed by this method. For analysis of (aqueous) test samples equal amounts of the solvent used in the calibration standards are added to the test samples. Thus, the test samples are treated in the same manner as the calibration samples and contained water and solvent at same concentrations (volume mixture methanol/water = 1+10).

This procedure was repeated on every measuring day and new matrix calibration functions were recorded.

Matrix calibration of the LC-MS/MS system and creating the calibration function

The LC-MS/MS system was calibrated for the analysis of the aqueous test samples by preparation and measuring of the prepared calibration samples (cp. previous chapters). Afterwards the chromatographic raw data were processed (integrated) using the Waters Quan-Lynx software. Subsequent the calibration functions were set up by the 'internal standard method' plotting the peak area ratios (PAR = integrated peak area analyte / integrated peak area IS) against the used analyte concentrations. With the received calibration data a linear regression calculation was performed.

Quantification and calculation of the analytical results

The LC-MS/MS quantification data were generated by processing the chromatographic raw data of the measured samples and by subsequent calculation of the quantification results ($C_{LC\text{-}MS/MS}$) using the respective matrix calibration function.

Calculation chironomid test water samples

As the aqueous test samples in the chironomid test (water) were diluted 1:1000 before measurement and were analyzed by direct injection into the LC-MS/MS system, the concentrations of the analyte in the aqueous test samples (CW) were quantified from the relevant calibration function ($C_{LC\text{-}MS/MS} = C_W$) by multiplication with a factor of 1000.

Calculation chironomid test sediment samples

The calculation of the concentration in sediment and the conversion from ng/L (measured value) to mg/kg (concentration in sediment) was performed as follows.

$C_{sediment} = C_{measured} * V_{acetone} * df / dw_{sediment} / 1000000$

Where:

$C_{sediment}$: concentration of analyte in sediment sample [mg/kg]

$C_{measured}$: measured concentration [ng/L]

V_{final}: final volume if the sample for analysis [mg]

$V_{acetone}$: Volume of acetone used for extraction [mL]

df: dilution factor

$dw_{sediment}$: dry weight of the sediment sample [g]

1000000: conversion factor to change the units from ng/kg to mg/kg

Calculation ZEOGRT test

As the aqueous test samples from the ZEOGRT test (water) and the calibration samples were pre-treated (diluted) in the same way and were analyzed by direct injection into the LC-MS/MS system, the concentrations of the analyte in the aqueous test samples (CW) were quantified directly from the relevant calibration function ($C_{LC\text{-}MS/MS}$ = CW).

Quality control

Two quality control (QC) standards were used for the verification of the basic calibration. The QC standard solutions were prepared analogous to calibration standards, but were based on separate weights of the analytical standard, cp. B.1.5 (solutions S-1b and S-1d).

The QC standard solutions QC-1b and QC-2b were prepared on September 07, 2018 in concentrations of 50.0 ng/L and 2500 ng/L by diluting the IM-2b solution in separate volumetric flasks with methanol; see pipetting plan in Table 6.

On the day of measurement, the QC samples QC-S-1b and QC-S-2b were prepared by mixing 100 µL of the QC standard solutions with 25.0 µL of IS solution IS-IM-2a and 1000 µL aqueous test medium in screw top vials (volume mixture water/methanol = 10+1 (v+v). The analyte concentration in the QC samples were thus 5.00 ng/L and 250 ng/L, the QC samples were measured in turn as described in chapter B.1.4.

The prepared QC standard solutions were stored in a refrigerator as well.

Table 6: Preparation of the quality control (QC) standard and QC samples.

No. of the QC standard	Volume solution IM-2b	Volumetric flask, nominal volume	Analyte concentration, QC solution	No. of the QC sample	Analyte concentration, QC sample*
QC-1b	100.0 µL	10 mL	50.0 ng/L	QC-S-1b	5.00 ng/L
QC-2b	5000 µL	10 mL	2500 ng/L	QC S-2b	250 ng/L

*) See remark Table 5.

The measurement intervals of the QC standards were every twentieth sample, but at least once a measurement day.

As already mentioned, new QC standards were produced on November 19, 2019 (QC-1d and QC-2d). According to the described procedure, new QC samples were prepared and measured using the solution IM-2d; the concentrations were again 5.00 ng/L and 250 ng/L.

B.1.6 Results

Matrix calibration function

For measurements of the test samples from the Chironomid test:

The basic calibration function (response type: internal standard) used for the quantification of Dienogest in the measured aqueous test medium samples is shown in Figure 1; the calibration function was calculated by linear regression analysis using the Waters QuanLynx software to:

Function: $PAR = 0.00839383 \bullet C_{Cal} + 0.00122893$ $r^2 = 0.999875$

PAR = Peak area ratio

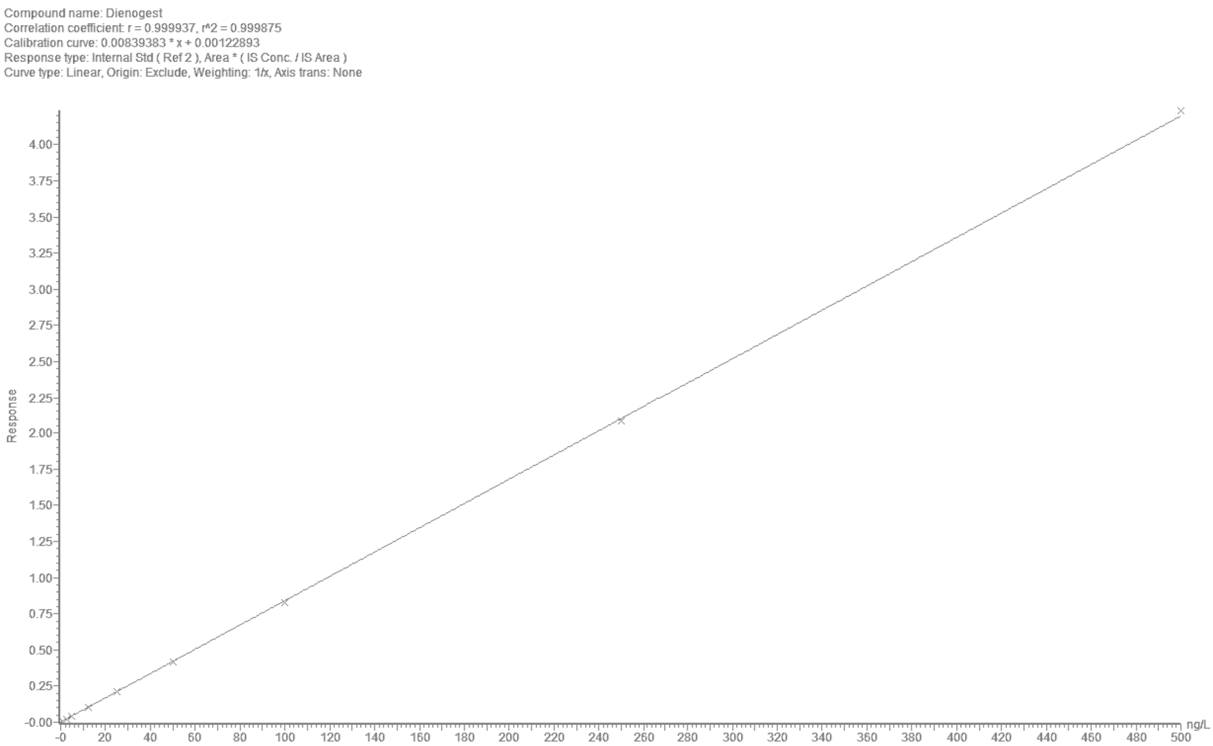
C_{Cal} = Analyte concentration of the calibration solutions

r^2 = Coefficient of determination

Linearity

Using the linear regression model, the coefficient of determination for Dienogest was calculated to be 0.999875. As the calculated r^2-values were very close to 1, the linearity of the calibration functions was accepted.

Figure 1: Basic calibration function of Dienogest as measured November 09, 2018 (single injection)



Source: Own graphic, Fraunhofer IME

For measurements of the test samples from the ZEOGRT test:

The basic calibration function (response type: internal standard) used for the quantification of Dienogest in the measured aqueous test medium samples is shown in Figure 2; the calibration function was calculated by linear regression analysis using the Waters QuanLynx software to:

Function: $PAR = 0.00814641 \bullet C_{Cal} + 0.000415304$ $r^2 = 0.999968$

PAR = Peak area ratio

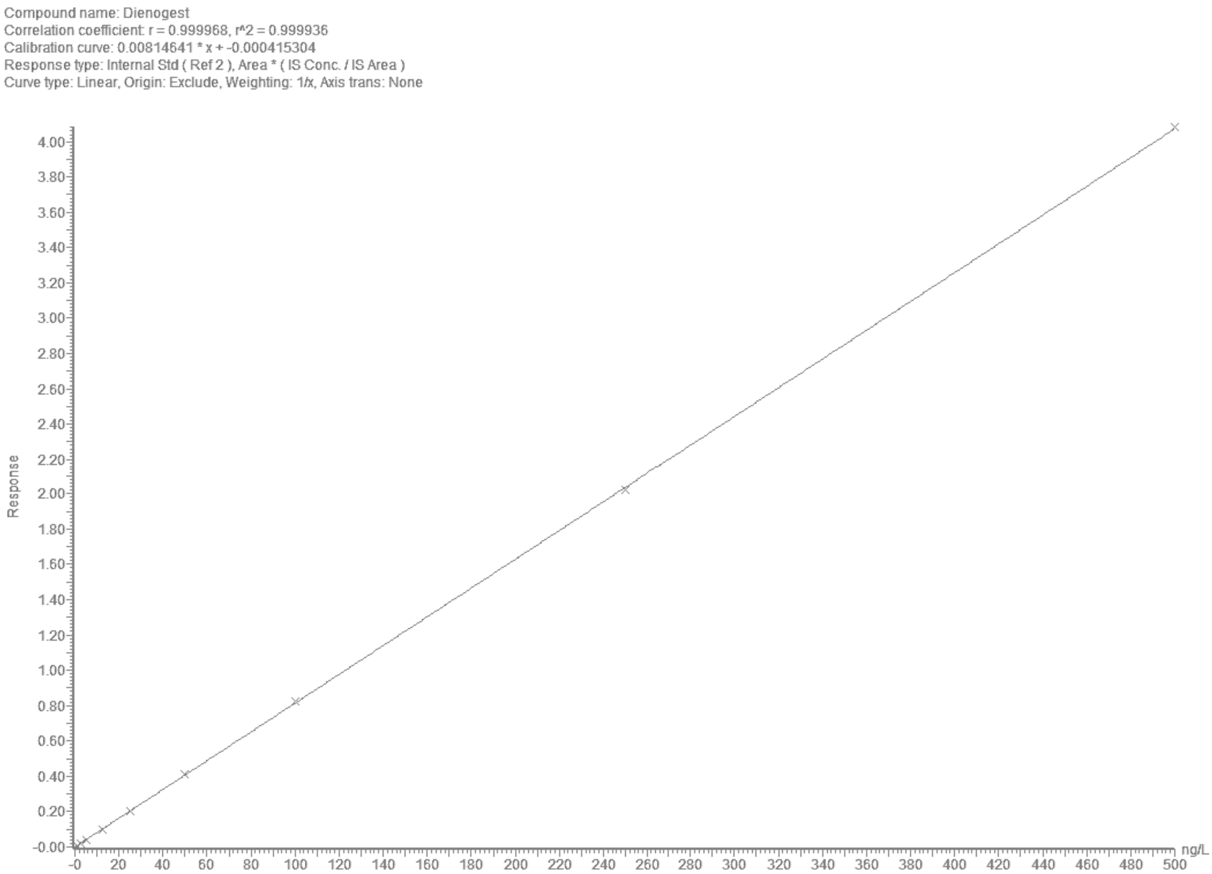
C_{Cal} = Analyte concentration of the calibration solutions

r^2 = Coefficient of determination

Linearity

Using the linear regression model, the coefficient of determination for Dienogest was calculated to be 0.999968. As the calculated r^2-values were very close to 1, the linearity of the calibration functions was accepted.

Figure 2: Basic calibration function of Dienogest as measured November 21, 2018 (single injection)



Source: Own graphic, Fraunhofer IME

Results of the analysed samples

Chironomid Life Cycle Test: The Dienogest concentrations in sediment in all treatment levels and controls were determined by chemical analysis at the start and in the two highest concentration levels at the end of the test. The Dienogest concentrations in aqueous test media samples were assessed by chemical analysis, samples of treatments and controls were analysed at the test start for the first generation, in the crystalline dish after 16 days, at test start for the second generation in the two highest concentration, at the test end for the first generation and the second generation respectively (28 days). Furthermore, the concentration was determined for the aqueous phase in the crystalline dish after seven days after day 0 of the second generation.

Table 7: Analyzed Dienogest concentrations (CW) in sediment and corresponding 'percent of nominal' values (mass transition m/z 312.28 m/z 160.98).

Sampling date (2018)	Treatment	Nominal Dienogest conc.	Dryweight sediment	LC-MS/MS quant. data cLC-MS/MS	Dilution factor	Measured analyte conc. Cw	Percent of nominal
October 24, 2018 (G1) 0d	Control		9.66 g	<LOQ*	10000	<LOQ	
	Conc.1	6.25 mg/kg	9.81 g	62.1 ng/L	10000	6.33 mg/kg	101%
	Conc.2	12.5 mg/kg	9.70 g	116 ng/L	10000	12.0 mg/kg	95.9%
	Conc.3	25 mg/kg	9.97 g	201 ng/L	10000	20.1 mg/kg	80.4%
	Conc.4	50 mg/kg	9.36 g	232 ng/L	20000	49.6 mg/kg	99.2%
	Conc.5	100 mg/kg	9.86 g	230 ng/L	40000	93.3 mg/kg	93.3%
November 19, 2018 (G2) 0d	Control		10.57 g	<LOQ*	10000	<LOQ	
	Conc.1	6.25 mg/kg	10.41 g	56.3 ng/L	10000	5.41 mg/kg	86.6%
	Conc.2	12.5 mg/kg	10.21 g	122 ng/L	10000	11.9 mg/kg	95.5%
	Conc.3	25 mg/kg	10.62 g	251 ng/L	10000	23.6 mg/kg	94.4%
	Conc.4	50 mg/kg	10.40 g	243 ng/L	20000	46.7 mg/kg	93.4%
	Conc.5	100 mg/kg	10.37 g	245 ng/L	40000	94.5 mg/kg	94.5%
November 21, 2018 (G1) 28d	Conc.4	50 mg/kg	9.49 g	195 ng/L	20000	41.1 mg/kg	82.2%
	Conc.5	100 mg/kg	9.48 g	196 ng/L	40000	82.8 mg/kg	82.8%
December 17, 2018 (G2) 28d	Conc.4	50 mg/kg	9.29 g	213 ng/L	20000	45.9 mg/kg	91.8%
	Conc.5	100 mg/kg	9.40 g	204 ng/L	40000	87.0 mg/kg	87.0%

*The LOQ was set at 1.5 ng/L as the measured concentration.

Table 8: Analyzed Dienogest concentrations (CW) in water (mass transition m/z 312.28 m/z 160.98).

Sampling date (2018)	Treatment	LC-MS/MS quant. data cLC-MS/MS	Dilution factor	Measured analyte conc. Cw
October 24, 2018	G1 0d Control	<LOQ*	1000	<LOQ
	G1 0d Conc.1	30.5 ng/L	1000	30.5 µg/L
	G1 0d Conc.2	63.9 ng/L	1000	63.9 µg/L
	G1 0d Conc.3	120 ng/L	1000	120 µg/L
	G1 0d Conc.4	330 ng/L	1000	330 µg/L
	G1 0d Conc.5	336 ng/L	2000	673 µg/L
November 09, 2018 crystalline dish	0d Control	<LOQ*	1000	<LOQ µg/L
	0d Conc.1	24.3 ng/L	1000	24.3 µg/L
	0d Conc.2	49.6 ng/L	1000	49.6 µg/L
	0d Conc.3	129 ng/L	1000	129 µg/L
	0d Conc.4	276 ng/L	1000	276 µg/L
	0d Conc.5	243 ng/L	2000	486 µg/L
November 19, 2018	G2 0d Control	<LOQ*	1000	<LOQ
	G2 0d Conc.1	23.5 ng/L	1000	23.5 µg/L
	G2 0d Conc.2	63.7 ng/L	1000	63.7 µg/L
	G2 0d Conc.3	159 ng/L	1000	159 µg/L
	G2 0d Conc.4	350 ng/L	1000	350 µg/L
	G2 0d Conc.5	408 ng/L	2000	815 µg/L
November 21, 2018	G1 28d Conc.4	281 ng/L	1000	281 µg/L
	G1 28d Conc.5	321 ng/L	2500	802 µg/L
November 26, 2018 crystalline dish	Conc.4	412 ng/L	1000	412 µg/L
	Conc.5	461 ng/L	2500	1153 µg/L
December 17, 2018	G2 28d Conc.4	314 ng/L	1000	314 µg/L
	G2 28d Conc.5	306 ng/L	2500	765 µg/L

*The LOQ was set at 1.5 ng/L as the measured concentration.

<u>ZEOGRT:</u> The Dienogest concentrations in the aqueous phase were determined in all replicates of all treatment levels and controls at test start. Thereafter, 2 replicates of each treatment level and control were sampled and analysed for each sampling time point.

Table 9 Analyzed Dienogest concentrations (CW) and corresponding 'percent of nominal' values (mass transition m/z 312.28 m/z 160.98).

Sampling date and time (2018, 2019)	Treatment	Test vessel	Nominal conc.	LC-MS/MS quant. data cLC-MS/MS	Measured analyte conc. Cw	Percent of nominal
September 25, 2018 8:30 am	Control	1		<LOQ*	<LOQ	
		2		<LOQ*	<LOQ	
		3		<LOQ*	<LOQ	
		4		<LOQ*	<LOQ	
	Conc. 1	1	3.20 ng/L	3.17 ng/L	3.17 ng/L	99.0%
		2		3.46 ng/L	3.46 ng/L	108%
		3		3.56 ng/L	3.56 ng/L	111%
		4		3.33 ng/L	3.33 ng/L	104%
	Conc. 2	1	10.0 ng/L	11.1 ng/L	11.1 ng/L	111%
		2		10.8 ng/L	10.8 ng/L	108%
		3		10.5 ng/L	10.5 ng/L	105%
		4		10.4 ng/L	10.4 ng/L	104%
	Conc. 3	1	32.0 ng/L	30.3 ng/L	30.3 ng/L	94.8%
		2		30.5 ng/L	30.5 ng/L	95.4%
		3		35.9 ng/L	35.9 ng/L	112%
		4		35.9 ng/L	35.9 ng/L	112%
	Conc. 4	1	100 ng/L	102 ng/L	102 ng/L	102%
		2		103 ng/L	103 ng/L	103%
		3		110 ng/L	110 ng/L	110%
		4		114 ng/L	114 ng/L	114%
	Conc. 5	1	320 ng/L	305 ng/L	305 ng/L	95.2%
		2		313 ng/L	313 ng/L	97.9%
		3		288 ng/L	288 ng/L	90.1%
		4		287 ng/L	287 ng/L	89.7%
October 1, 2018 8:30 am	Control	1		<LOQ*	<LOQ	
		3		<LOQ*	<LOQ	
	Conc. 1	1	3.20 ng/L	9.83 ng/L	9.83 ng/L	307%
		3		12.1 ng/L	12.1 ng/L	379%

Sampling date and time (2018, 2019)	Treatment	Test vessel	Nominal conc.	LC-MS/MS quant. data cLC-MS/MS	Measured analyte conc. Cw	Percent of nominal
	Conc. 2	1	10.0 ng/L	21.5 ng/L	21.5 ng/L	215%
		3		19.5 ng/L	19.5 ng/L	195%
	Conc. 3	1	32.0 ng/L	59.6 ng/L	59.6 ng/L	186%
		3		66.9 ng/L	66.9 ng/L	209%
	Conc. 4	1	100 ng/L	93.4 ng/L	93.4 ng/L	93.4%
		3		95.8 ng/L	95.8 ng/L	95.8%
	Conc. 5	1	320 ng/L	254 ng/L	254 ng/L	79.3%
		3		256 ng/L	256 ng/L	79.9%
October 2, 2018 8:10 am	Control	2		2.65 ng/L	2.65 ng/L	
		4		0.922 ng/L	<LOQ	
	Conc. 1	2	3.20 ng/L	4.92 ng/L	4.92 ng/L	154%
		4		5.05 ng/L	5.05 ng/L	158%
	Conc. 2	2	10.0 ng/L	11.8 ng/L	11.8 ng/L	118%
		4		9.59 ng/L	9.59 ng/L	95.9%
	Conc. 3	2	32.0 ng/L	32.1 ng/L	32.1 ng/L	100%
		4		34.5 ng/L	34.5 ng/L	108%
	Conc. 4	2	100 ng/L	104 ng/L	104 ng/L	104%
		4		108 ng/L	108 ng/L	108%
	Conc. 5	2	320 ng/L	349 ng/L	349 ng/L	109%
		4		311 ng/L	311 ng/L	97.3%
October 8, 2018 8:50 am	Control	1		<LOQ*	<LOQ	
		3		<LOQ*	<LOQ	
	Conc. 1	1	3.20 ng/L	2.82 ng/L	2.82 ng/L	88.1%
		3		2.76 ng/L	2.76 ng/L	86.1%
	Conc. 2	1	10.0 ng/L	8.32 ng/L	8.32 ng/L	83.2%
		3		7.85 ng/L	7.85 ng/L	78.5%
	Conc. 3	1	32.0 ng/L	25.9 ng/L	25.9 ng/L	80.9%
		3		29.6 ng/L	29.6 ng/L	92.7%
	Conc. 4	1	100 ng/L	106 ng/L	106 ng/L	106%
		3		117 ng/L	117 ng/L	117%

Sampling date and time (2018, 2019)	Treatment	Test vessel	Nominal conc.	LC-MS/MS quant. data cLC-MS/MS	Measured analyte conc. Cw	Percent of nominal
	Conc. 5	1	320 ng/L	335 ng/L	335 ng/L	105%
		3		344 ng/L	344 ng/L	107%
October 15, 2018 9:40 am	Control	2		<LOQ*	<LOQ	
		4		<LOQ*	<LOQ	
	Conc. 1	2	3.20 ng/L	2.48 ng/L	2.48 ng/L	77.6%
		4		2.33 ng/L	2.33 ng/L	72.8%
	Conc. 2	2	10.0 ng/L	8.28 ng/L	8.28 ng/L	82.8%
		4		8.37 ng/L	8.37 ng/L	83.7%
	Conc. 3	2	32.0 ng/L	23.6 ng/L	23.6 ng/L	73.9%
		4		24.9 ng/L	24.9 ng/L	77.8%
	Conc. 4	2	100 ng/L	96.2 ng/L	96.2 ng/L	96.2%
		4		107 ng/L	107 ng/L	107%
	Conc. 5	2	320 ng/L	347 ng/L	347 ng/L	108%
		4		318 ng/L	318 ng/L	99.5%
October 23, 2018 8:50 am	Control	1		<LOQ*	<LOQ	
		3		<LOQ*	<LOQ	
	Conc. 1	1	3.20 ng/L	2.07 ng/L	2.07 ng/L	64.6%
		3		1.72 ng/L	1.72 ng/L	53.8%
	Conc. 2	1	10.0 ng/L	5.57 ng/L	5.57 ng/L	55.7%
		3		5.44 ng/L	5.44 ng/L	54.4%
	Conc. 3	1	32.0 ng/L	14.5 ng/L	14.5 ng/L	45.4%
		3		16.9 ng/L	16.9 ng/L	52.8%
	Conc. 4	1	100 ng/L	50.1 ng/L	50.1 ng/L	50.1%
		3		64.1 ng/L	64.1 ng/L	64.1%
	Conc. 5	1	320 ng/L	175 ng/L	175 ng/L	54.6%
		3		191 ng/L	191 ng/L	59.7%
October 29, 2018 9:20 am	Control	2		<LOQ*	<LOQ	
		4		<LOQ*	<LOQ	
	Conc. 1	2	3.20 ng/L	2.69 ng/L	2.69 ng/L	83.9%

Sampling date and time (2018, 2019)	Treatment	Test vessel	Nominal conc.	LC-MS/MS quant. data cLC-MS/MS	Measured analyte conc. Cw	Percent of nominal
		4		2.69 ng/L	2.69 ng/L	84.0%
	Conc. 2	2	10.0 ng/L	8.14 ng/L	8.14 ng/L	81.4%
		4		8.61 ng/L	8.61 ng/L	86.1%
	Conc. 3	2	32.0 ng/L	22.5 ng/L	22.5 ng/L	70.4%
		4		24.2 ng/L	24.2 ng/L	75.7%
	Conc. 4	2	100 ng/L	67.7 ng/L	67.7 ng/L	67.7%
		4		99.4 ng/L	99.4 ng/L	99.4%
	Conc. 5	2	320 ng/L	285 ng/L	285 ng/L	89.0%
		4		298 ng/L	298 ng/L	93.0%
November 06, 2018 8:25 am	Control	1		<LOQ*	<LOQ	
		3		<LOQ*	<LOQ	
	Conc. 1	1	3.20 ng/L	2.33 ng/L	2.33 ng/L	72.8%
		3		2.29 ng/L	2.29 ng/L	71.4%
	Conc. 2	1	10.0 ng/L	10.1 ng/L	10.1 ng/L	101%
		3		8.68 ng/L	8.68 ng/L	86.8%
	Conc. 3	1	32.0 ng/L	20.7 ng/L	20.7 ng/L	64.7%
		3		27.3 ng/L	27.3 ng/L	85.3%
	Conc. 4	1	100 ng/L	71.5 ng/L	71.5 ng/L	71.5%
		3		103 ng/L	103 ng/L	103%
	Conc. 5	1	320 ng/L	262 ng/L	262 ng/L	81.8%
		3		314 ng/L	314 ng/L	98.2%
November 12, 2018 8:45 am	Control	2		<LOQ*	<LOQ	
		4		<LOQ*	<LOQ	
	Conc. 1	2	3.20 ng/L	2.78 ng/L	2.78 ng/L	86.7%
		4		3.06 ng/L	3.06 ng/L	95.5%
	Conc. 2	2	10.0 ng/L	9.55 ng/L	9.55 ng/L	95.5%
		4		9.70 ng/L	9.70 ng/L	97.0%
	Conc. 3	2	32.0 ng/L	29.3 ng/L	29.3 ng/L	91.6%
		4		33.5 ng/L	33.5 ng/L	105%
	Conc. 4	2	100 ng/L	104 ng/L	104 ng/L	104%

Sampling date and time (2018, 2019)	Treatment	Test vessel	Nominal conc.	LC-MS/MS quant. data cLC-MS/MS	Measured analyte conc. Cw	Percent of nominal
		4		97.4 ng/L	97.4 ng/L	97.4%
	Conc. 5	2	320 ng/L	320 ng/L	320 ng/L	99.9%
		4		295 ng/L	295 ng/L	92.1%
November 20, 2018 9:10 am	Control	1		<LOQ*	<LOQ	
		3		<LOQ*	<LOQ	
	Conc. 1	1	3.20 ng/L	3.09 ng/L	3.09 ng/L	96.6%
		3		2.87 ng/L	2.87 ng/L	89.5%
	Conc. 2	1	10.0 ng/L	11.0 ng/L	11.0 ng/L	110%
		3		9.37 ng/L	9.37 ng/L	93.7%
	Conc. 3	1	32.0 ng/L	30.6 ng/L	30.6 ng/L	95.5%
		3		33.0 ng/L	33.0 ng/L	103%
	Conc. 4	1	100 ng/L	114 ng/L	114 ng/L	114%
		3		114 ng/L	114 ng/L	114%
	Conc. 5	1	320 ng/L	322 ng/L	322 ng/L	101%
		3		353 ng/L	353 ng/L	110%
November 26, 2018 8:45 am	Control	2		<LOQ*	<LOQ	
		4		<LOQ*	<LOQ	
	Conc. 1	2	3.20 ng/L	3.03 ng/L	3.03 ng/L	94.5%
		4		2.73 ng/L	2.73 ng/L	85.4%
	Conc. 2	2	10.0 ng/L	9.27 ng/L	9.27 ng/L	92.7%
		4		9.70 ng/L	9.70 ng/L	97.0%
	Conc. 3	2	32.0 ng/L	32.6 ng/L	32.6 ng/L	102%
		4		34.8 ng/L	34.8 ng/L	109%
	Conc. 4	2	100 ng/L	89.9 ng/L	89.9 ng/L	89.9%
		4		105 ng/L	105 ng/L	105%
	Conc. 5	2	320 ng/L	337 ng/L	337 ng/L	105%
		4		346 ng/L	346 ng/L	108%
December 05, 2018 9:20 am	Control	1		<LOQ*	<LOQ	
		3		<LOQ*	<LOQ	

Sampling date and time (2018, 2019)	Treatment	Test vessel	Nominal conc.	LC-MS/MS quant. data cLC-MS/MS	Measured analyte conc. Cw	Percent of nominal
	Conc. 1	1	3.20 ng/L	3.09 ng/L	3.09 ng/L	96.6%
		3		2.76 ng/L	2.76 ng/L	86.2%
	Conc. 2	1	10.0 ng/L	9.32 ng/L	9.32 ng/L	93.2%
		3		9.25 ng/L	9.25 ng/L	92.5%
	Conc. 3	1	32.0 ng/L	29.3 ng/L	29.3 ng/L	91.7%
		3		30.5 ng/L	30.5 ng/L	95.5%
	Conc. 4	1	100 ng/L	108 ng/L	108 ng/L	108%
		3		104 ng/L	104 ng/L	104%
	Conc. 5	1	320 ng/L	337 ng/L	337 ng/L	105%
		3		358 ng/L	358 ng/L	112%
December 10, 2018 9:00 am	Control	2		<LOQ*	<LOQ	
		4		<LOQ*	<LOQ	
	Conc. 1	2	3.20 ng/L	2.92 ng/L	2.92 ng/L	91.3%
		4		3.09 ng/L	3.09 ng/L	96.6%
	Conc. 2	2	10.0 ng/L	9.39 ng/L	9.39 ng/L	93.9%
		4		8.58 ng/L	8.58 ng/L	85.8%
	Conc. 3	2	32.0 ng/L	28.7 ng/L	28.7 ng/L	89.5%
		4		31.7 ng/L	31.7 ng/L	99.0%
	Conc. 4	2	100 ng/L	116 ng/L	116 ng/L	116%
		4		114 ng/L	114 ng/L	114%
	Conc. 5	2	320 ng/L	358 ng/L	358 ng/L	112%
		4		411 ng/L	411 ng/L	128%
December 17, 2018 9:30 am	Control	1		<LOQ*	<LOQ	
		3		<LOQ*	<LOQ	
	Conc. 1	1	3.20 ng/L	3.66 ng/L	3.66 ng/L	114%
		3		3.81 ng/L	3.81 ng/L	119%
	Conc. 2	1	10.0 ng/L	10.6 ng/L	10.6 ng/L	106%
		3		10.3 ng/L	10.3 ng/L	103%
	Conc. 3	1	32.0 ng/L	31.9 ng/L	31.9 ng/L	100%
		3		33.6 ng/L	33.6 ng/L	105%

Sampling date and time (2018, 2019)	Treatment	Test vessel	Nominal conc.	LC-MS/MS quant. data cLC-MS/MS	Measured analyte conc. Cw	Percent of nominal
	Conc. 4	1	100 ng/L	120 ng/L	120 ng/L	120%
		3		127 ng/L	127 ng/L	127%
	Conc. 5	1	320 ng/L	364 ng/L	364 ng/L	114%
		3		351 ng/L	351 ng/L	110%
December 27, 2018 10:30 am	Control	2		<LOQ*	<LOQ	
		4		<LOQ*	<LOQ	
	Conc. 1	2	3.20 ng/L	3.55 ng/L	3.55 ng/L	111%
		4		3.96 ng/L	3.96 ng/L	124%
	Conc. 2	2	10.0 ng/L	12.1 ng/L	12.1 ng/L	121%
		4		12.7 ng/L	12.7 ng/L	127%
	Conc. 3	2	32.0 ng/L	30.8 ng/L	30.8 ng/L	96.4%
		4		37.2 ng/L	37.2 ng/L	116%
	Conc. 4	2	100 ng/L	109 ng/L	109 ng/L	109%
		4		116 ng/L	116 ng/L	116%
	Conc. 5	2	320 ng/L	375 ng/L	375 ng/L	117%
		4		411 ng/L	411 ng/L	128%
January 03, 2019 8:45 am	Control	1		<LOQ*	<LOQ	
		3		<LOQ*	<LOQ	
	Conc. 1	1	3.20 ng/L	3.15 ng/L	3.15 ng/L	98.5%
		3		3.37 ng/L	3.37 ng/L	105%
	Conc. 2	1	10.0 ng/L	10.7 ng/L	10.7 ng/L	107%
		3		10.4 ng/L	10.4 ng/L	104%
	Conc. 3	1	32.0 ng/L	29.8 ng/L	29.8 ng/L	93.0%
		3		29.5 ng/L	29.5 ng/L	92.2%
	Conc. 4	1	100 ng/L	98.6 ng/L	98.6 ng/L	98.6%
		3		118 ng/L	118 ng/L	118%
	Conc. 5	1	320 ng/L	368 ng/L	368 ng/L	115%
		3		379 ng/L	379 ng/L	118%
January 07, 2019 9:10 am	Control	2		<LOQ*	<LOQ	

Sampling date and time (2018, 2019)	Treatment	Test vessel	Nominal conc.	LC-MS/MS quant. data cLC-MS/MS	Measured analyte conc. Cw	Percent of nominal
		4		<LOQ*	<LOQ	
	Conc. 1	2	3.20 ng/L	3.38 ng/L	3.38 ng/L	106%
		4		3.23 ng/L	3.23 ng/L	101%
	Conc. 2	2	10.0 ng/L	9.62 ng/L	9.62 ng/L	96.2%
		4		10.3 ng/L	10.3 ng/L	103%
	Conc. 3	2	32.0 ng/L	25.1 ng/L	25.1 ng/L	78.4%
		4		32.0 ng/L	32.0 ng/L	99.9%
	Conc. 4	2	100 ng/L	109 ng/L	109 ng/L	109%
		4		112 ng/L	112 ng/L	112%
	Conc. 5	2	320 ng/L	361 ng/L	361 ng/L	113%
		4		354 ng/L	354 ng/L	111%
January 14, 2019 9:00 am	Control	1		<LOQ*	<LOQ	
		3		<LOQ*	<LOQ	
	Conc. 1	1	3.20 ng/L	3.62 ng/L	3.62 ng/L	113%
		3		3.82 ng/L	3.82 ng/L	119%
	Conc. 2	1	10.0 ng/L	10.7 ng/L	10.7 ng/L	107%
		3		11.0 ng/L	11.0 ng/L	110%
	Conc. 3	1	32.0 ng/L	34.1 ng/L	34.1 ng/L	107%
		3		38.3 ng/L	38.3 ng/L	120%
	Conc. 4	1	100 ng/L	116 ng/L	116 ng/L	116%
		3		122 ng/L	122 ng/L	122%
	Conc. 5	1	320 ng/L	388 ng/L	388 ng/L	121%
		3		411 ng/L	411 ng/L	128%
January 21, 2019 8:50 am	Control	2		<LOQ*	<LOQ	
		4		<LOQ*	<LOQ	
	Conc. 1	2	3.20 ng/L	3.01 ng/L	3.01 ng/L	94.0%
		4		3.57 ng/L	3.57 ng/L	112%
	Conc. 2	2	10.0 ng/L	11.1 ng/L	11.1 ng/L	111%
		4		10.6 ng/L	10.6 ng/L	106%
	Conc. 3	2	32.0 ng/L	31.8 ng/L	31.8 ng/L	99.3%

Sampling date and time (2018, 2019)	Treatment	Test vessel	Nominal conc.	LC-MS/MS quant. data cLC-MS/MS	Measured analyte conc. Cw	Percent of nominal
		4		34.4 ng/L	34.4 ng/L	107%
	Conc. 4	2	100 ng/L	111 ng/L	111 ng/L	111%
		4		127 ng/L	127 ng/L	127%
	Conc. 5	2	320 ng/L	388 ng/L	388 ng/L	121%
		4		444 ng/L	444 ng/L	139%
January 28, 2019 8:45 am	Control	1		<LOQ*	<LOQ	
		3		<LOQ*	<LOQ	
	Conc. 1	1	3.20 ng/L	3.39 ng/L	3.39 ng/L	106%
		3		3.49 ng/L	3.49 ng/L	109%
	Conc. 2	1	10.0 ng/L	12.3 ng/L	12.3 ng/L	123%
		3		11.0 ng/L	11.0 ng/L	110%
	Conc. 3	1	32.0 ng/L	35.3 ng/L	35.3 ng/L	110%
		3		41.0 ng/L	41.0 ng/L	128%
	Conc. 4	1	100 ng/L	109 ng/L	109 ng/L	109%
		3		138 ng/L	138 ng/L	138%
	Conc. 5	1	320 ng/L	386 ng/L	386 ng/L	121%
		3		421 ng/L	421 ng/L	132%

*The LOQ was set at 1.5 ng/L as the measured concentration.

Daphnia magna reproduction test: All 42 samples were analysed. Results are shown in the table below.

Table 10: Analyzed Dienogest concentrations (CW) in water (mass transition m/z 312.28 m/z 160.98).

Sampling date	test vessel	nominal conc.	dilution factor	LC-MS/MS quant. data cLC-MS/MS	Measured analyte conc. cw	Percent of nominal
October 17, 2018 day 0 fresh	solvent control		2	<LOQ*	<LOQ	-
	control		2	<LOQ*	<LOQ	-
	conc. 1	12.8 µg/L	1000	11.4 ng/L	11.4 µg/L	89.3%
	conc. 2	32.0 µg/L	1000	29.2 ng/L	29.2 µg/L	91.3%
	conc. 3	80.0 µg/L	1000	72.4 ng/L	72.4 µg/L	90.4%
	conc. 4	200 µg/L	1000	184 ng/L	184 µg/L	91.9%
	conc. 5	500 µg/L	1000	459 ng/L	459 µg/L	91.8%
October 19, 2018 day 2 expo.	solvent control		2	<LOQ*	<LOQ	
	control		2	<LOQ*	<LOQ	
	conc. 1	12.8 µg/L	1000	11.8 ng/L	11.8 µg/L	92.1%
	conc. 2	32.0 µg/L	1000	28.8 ng/L	28.8 µg/L	90.0%
	conc. 3	80.0 µg/L	1000	72.7 ng/L	72.7 µg/L	90.9%
	conc. 4	200 µg/L	1000	179 ng/L	179 µg/L	89.4%
	conc. 5	500 µg/L	1000	464 ng/L	464 µg/L	92.9%
October 29, 2018 day 9 fresh	solvent control		2	<LOQ*	<LOQ	
	control		2	<LOQ*	<LOQ	
	conc. 1	12.8 µg/L	1000	13.0 ng/L	13.0 µg/L	101.9%
	conc. 2	32.0 µg/L	1000	31.1 ng/L	31.1 µg/L	97.1%
	conc. 3	80.0 µg/L	1000	81.1 ng/L	81.1 µg/L	101.4%
	conc. 4	200 µg/L	1000	204 ng/L	204 µg/L	102.2%
	conc. 5	500 µg/L	1000	517 ng/L	517 µg/L	103.4%
October 29, 2018 day 12 expo.	solvent control		2	<LOQ*	<LOQ	
	control		2	<LOQ*	<LOQ	

Sampling date	test vessel	nominal conc.	dilution factor	LC-MS/MS quant. data cLC-MS/MS	Measured analyte conc. cw	Percent of nominal
	conc. 1	12.8 µg/L	1000	12.1 ng/L	12.1 µg/L	94.4%
	conc. 2	32.0 µg/L	1000	29.9 ng/L	29.9 µg/L	93.6%
	conc. 3	80.0 µg/L	1000	74.3 ng/L	74.3 µg/L	92.8%
	conc. 4	200 µg/L	1000	190 ng/L	190 µg/L	95.2%
	conc. 5	500 µg/L	1000	451 ng/L	451 µg/L	90.2%
November 05, 2018 day 19 fresh	solvent control		2	0.00 ng/L	<LOQ	
	control		2	<LOQ*	<LOQ	
	conc. 1	12.8 µg/L	1000	12.4 ng/L	12.4 µg/L	96.5%
	conc. 2	32.0 µg/L	1000	29.1 ng/L	29.1 µg/L	91.1%
	conc. 3	80.0 µg/L	1000	78.1 ng/L	78.1 µg/L	97.6%
	conc. 4	200 µg/L	1000	194 ng/L	194 µg/L	96.8%
	conc. 5	500 µg/L	1000	480 ng/L	480 µg/L	96.0%
November 07, 2018 day 21 expo.	solvent control		2	0.81 ng/L	<LOQ	
	control		2	<LOQ*	<LOQ	
	conc. 1	12.8 µg/L	1000	12.8 ng/L	12.8 µg/L	100%
	conc. 2	32.0 µg/L	1000	29.6 ng/L	29.6 µg/L	92.5%
	conc. 3	80.0 µg/L	1000	72.3 ng/L	72.3 µg/L	90.3%
	conc. 4	200 µg/L	1000	179 ng/L	179 µg/L	89.4%
	conc. 5	500 µg/L	1000	436 ng/L	436 µg/L	87.2%

*The LOQ was set at 1.5 ng/L as the measured concentration.

Quality control

Quality controls had to be in the range of 80-120% for the calibration to be considered applicable for the quantification of the samples. Furthermore, the results of quality control showed that the calibration standards (calibration solutions) are stable over the period of approximately one year.

B.1.7 Representative LC-MS/MS chromatograms

Typical LC-MS/MS chromatograms of calibration samples, controls and test media samples are shown in Figure 3 to Figure 20. Each figure shows four ion chromatograms in stacked windows (top-down):

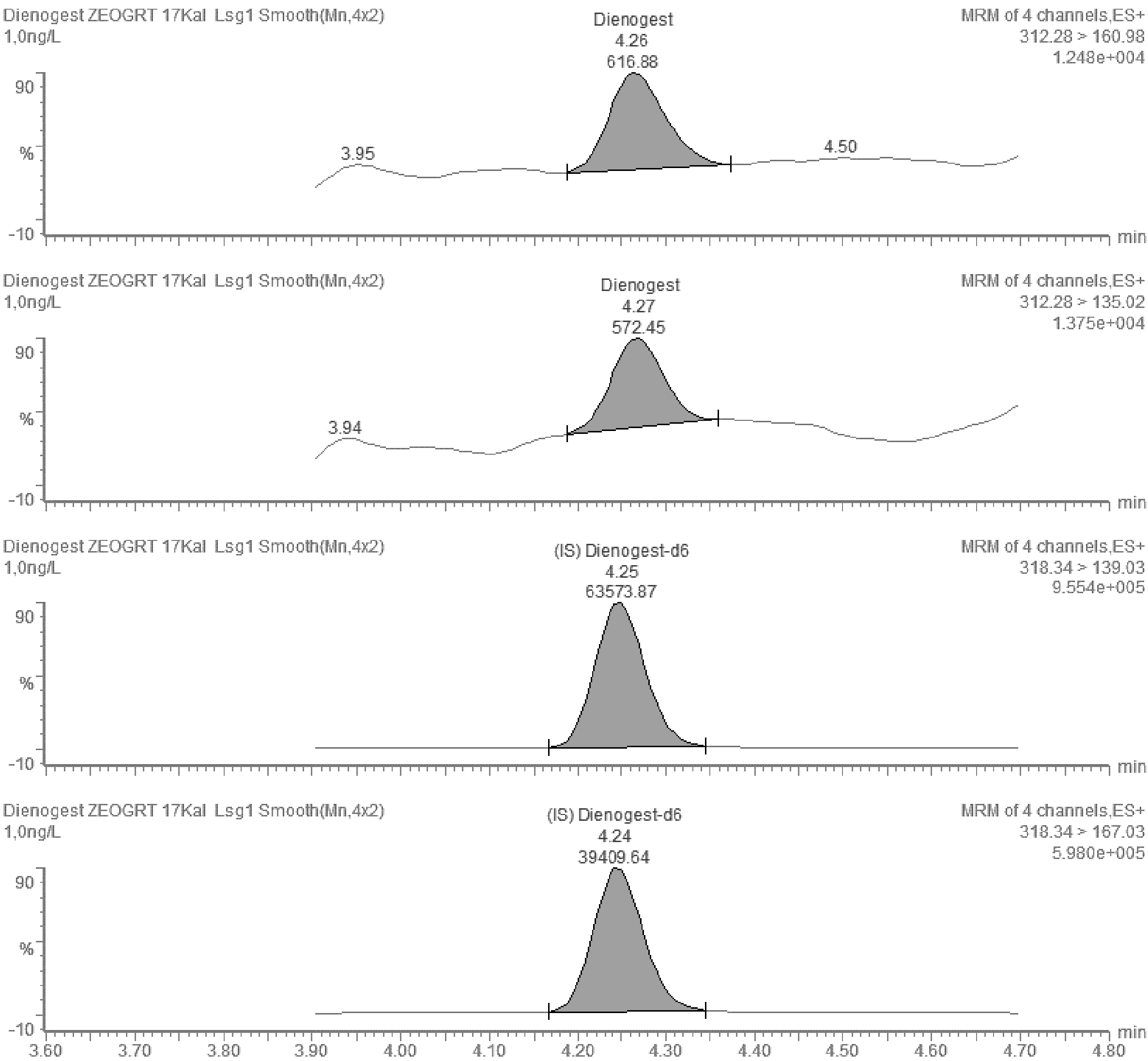
Ion chromatogram	Mass transition
Dienogest, quantification ion	m/z 312.28 → m/z 160.98
Dienogest, qualifier ion	m/z 312.28 → m/z 135.02
Dienogest-d6 (IS), quantification ion	m/z 318.34 → m/z 139.03
Dienogest-d6 (IS), qualifier ion	m/z 318.34 → m/z 167.03

The dashed line in some chromatograms shows the baseline of the integrated peaks executed by automatic integration using the Waters QuanLynx software. However, the grey highlighted part of the chromatographic peak reflects the manually integrated peak area; this corrected peak area was used for quantification of the analyte.

The retention time (tR) for Dienogest was approximately 4.25 min.

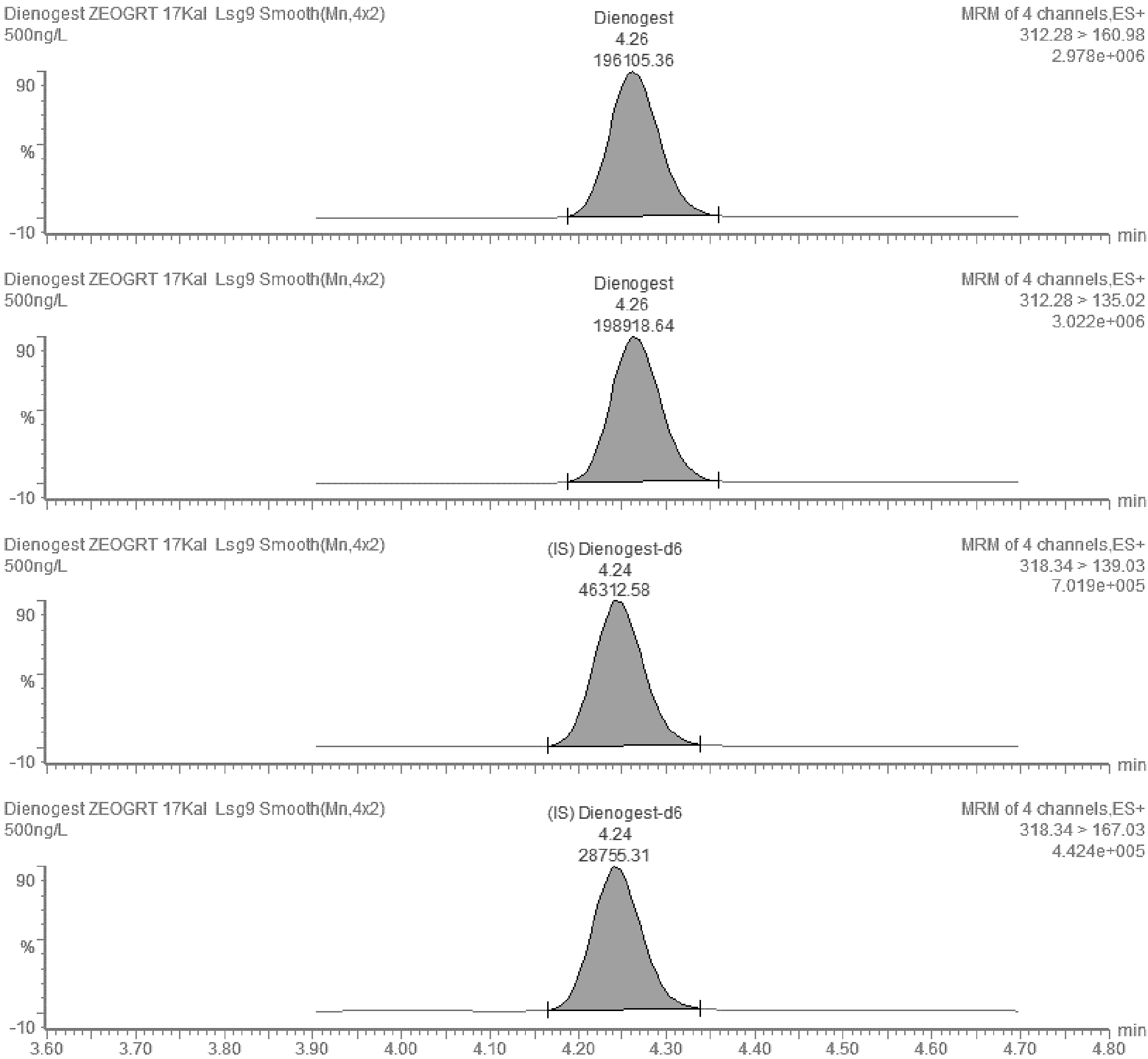
<u>Chironomid Life cycle test</u>

Figure 3: Calibration sample CS 1 measured November 09, 2018; Dienogest conc.: 1.00 ng/L.



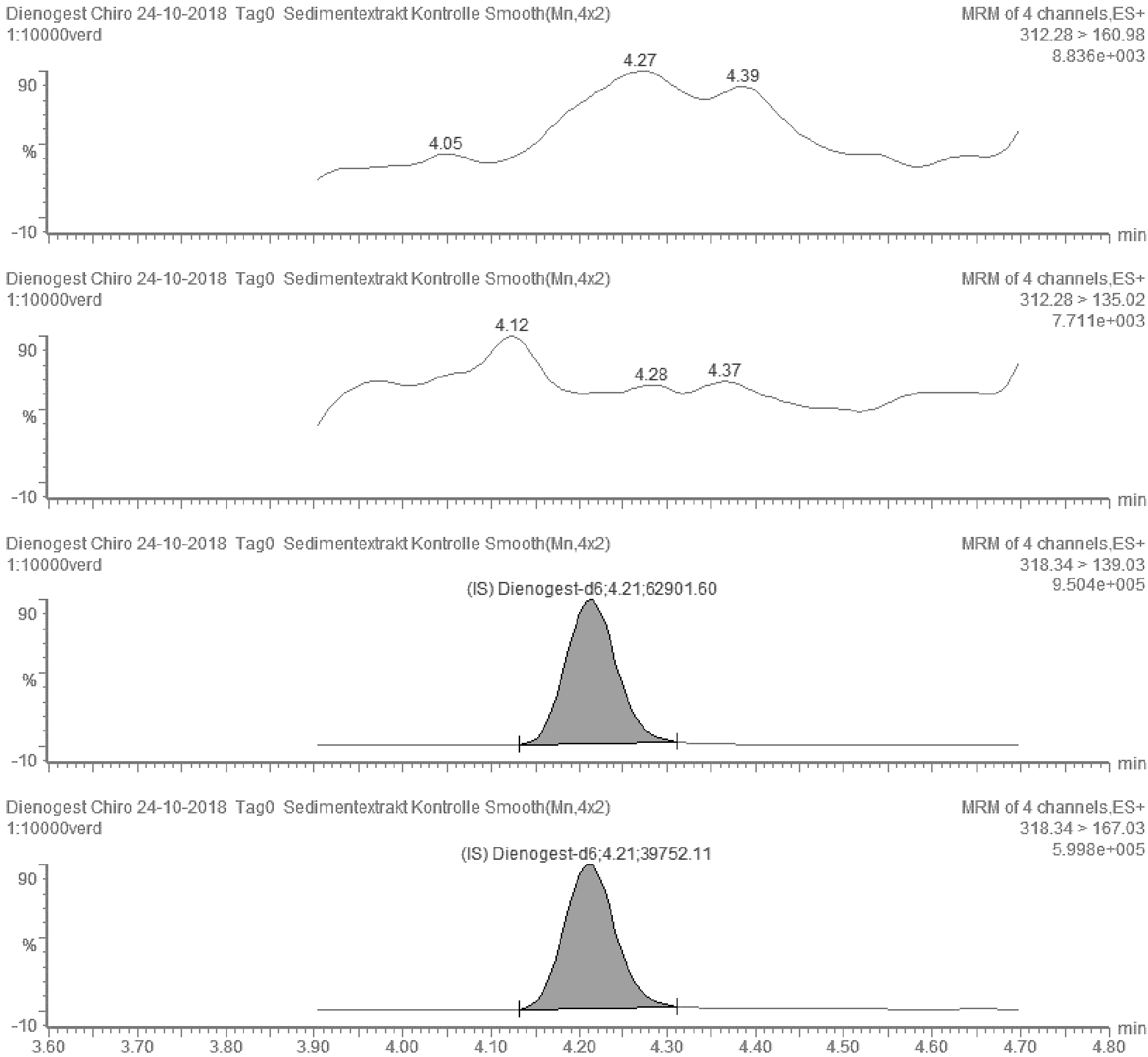
Source: Own graphic, Fraunhofer IME

Figure 4: Calibration sample CS 9 measured November 09, 2018; Dienogest conc.: 500 ng/L.



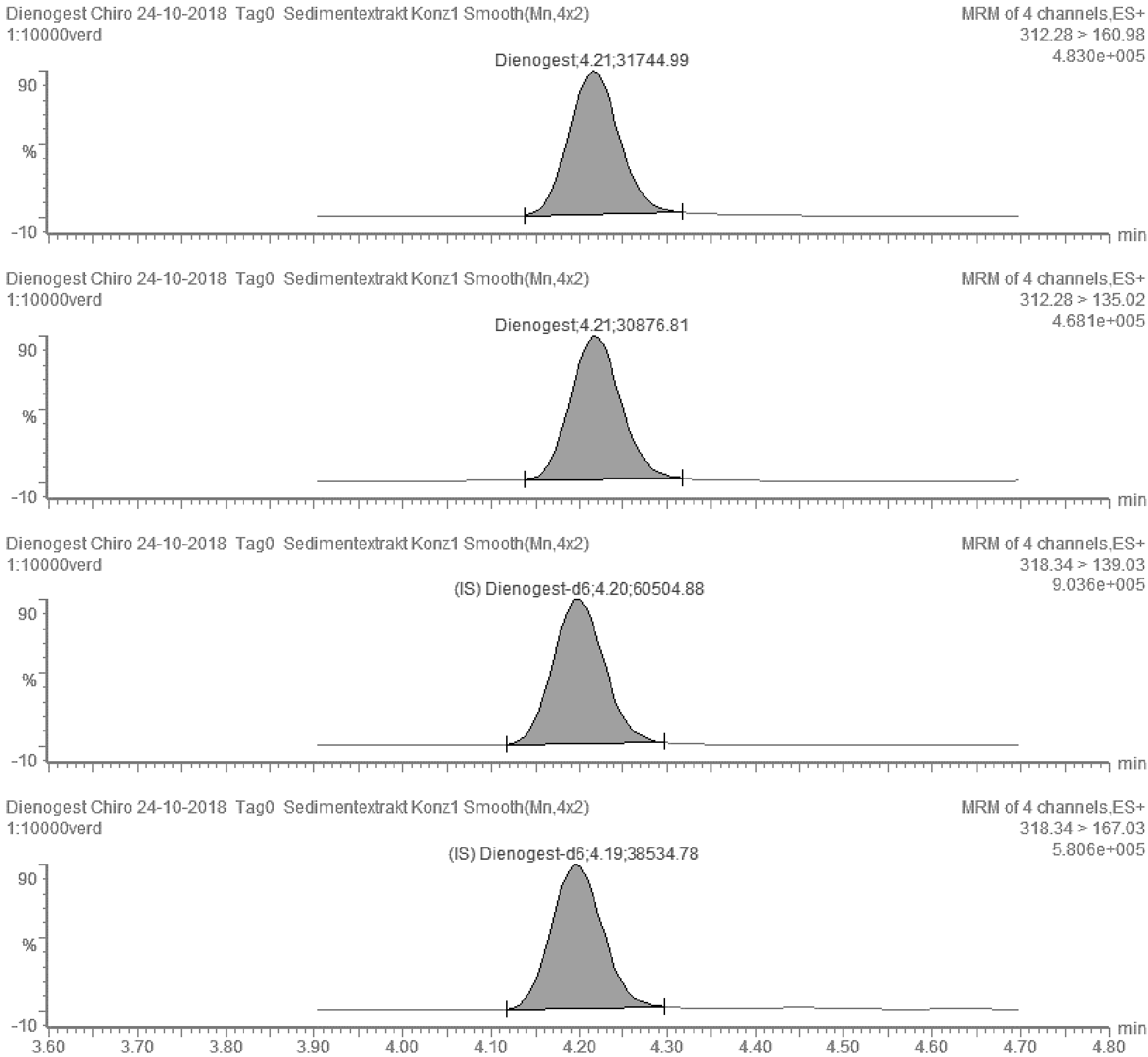
Source: Own graphic, Fraunhofer IME

Figure 5: Control sample, sediment, sampling time: test start (October 24, 2018).



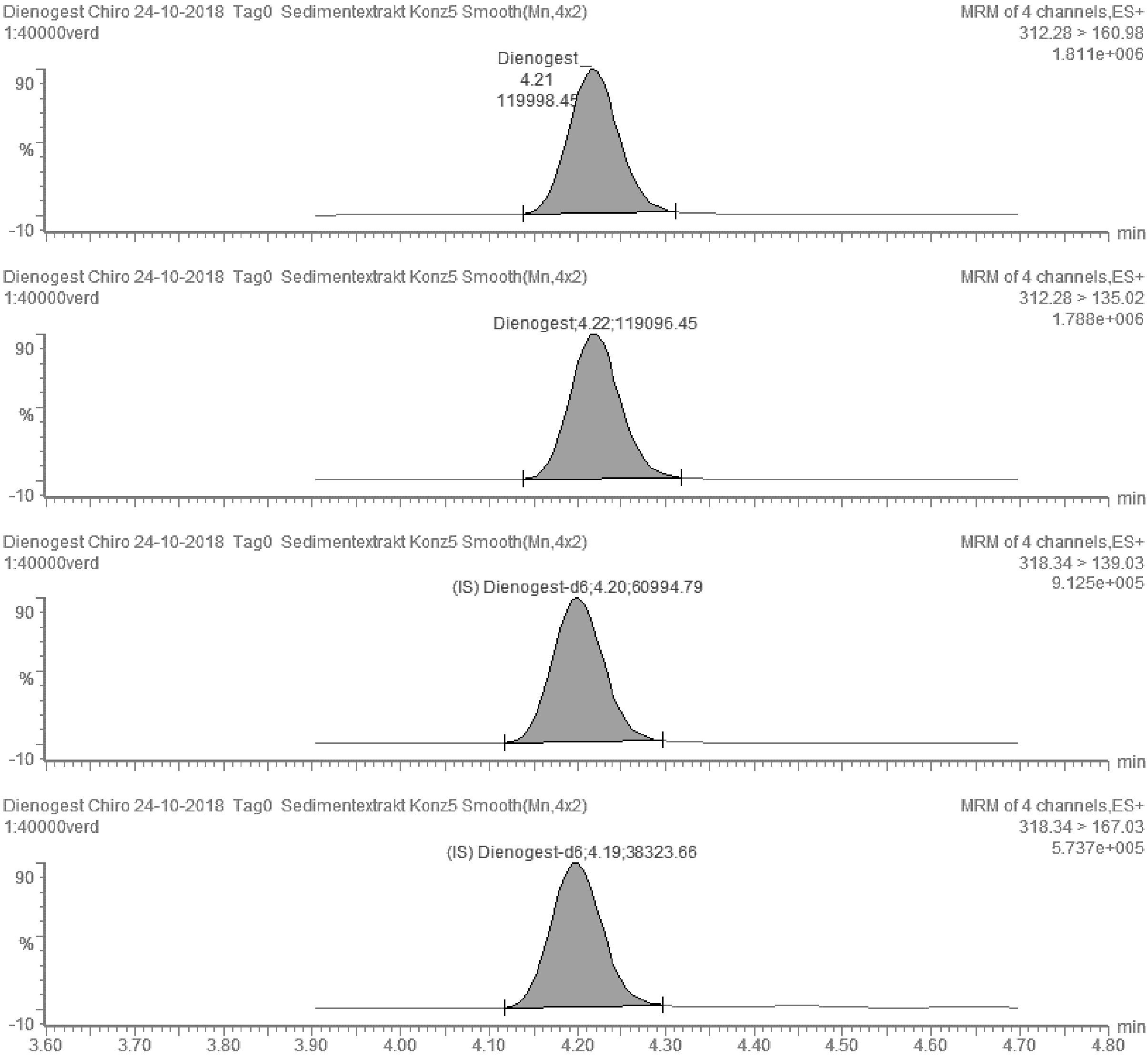
Source: Own graphic, Fraunhofer IME

Figure 6: Test sample concentration level 1, sediment, test start (October 24, 2018).



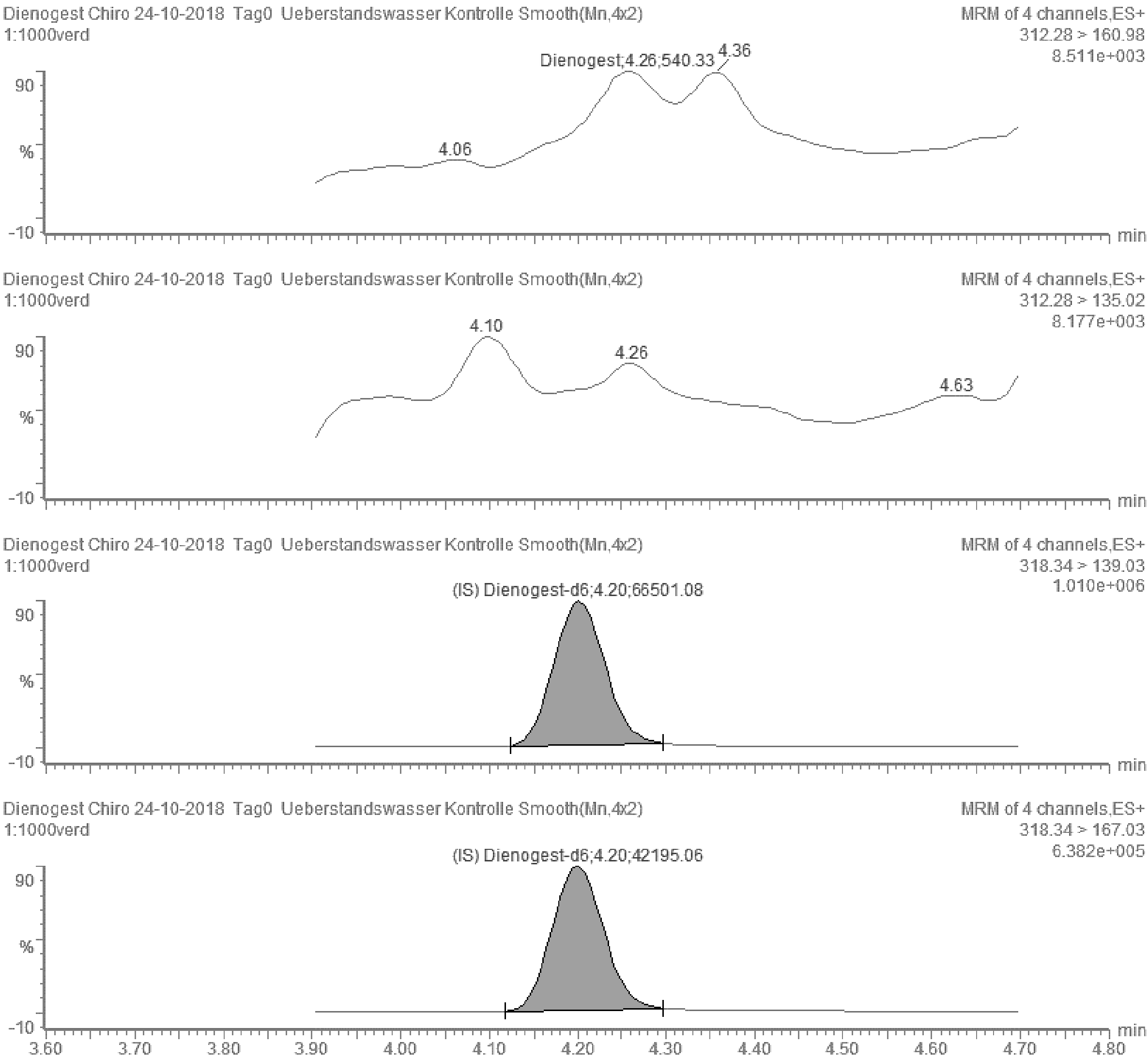
Source: Own graphic, Fraunhofer IME

Figure 7: Test sample concentration level 5, sediment, test start (October 24, 2018).



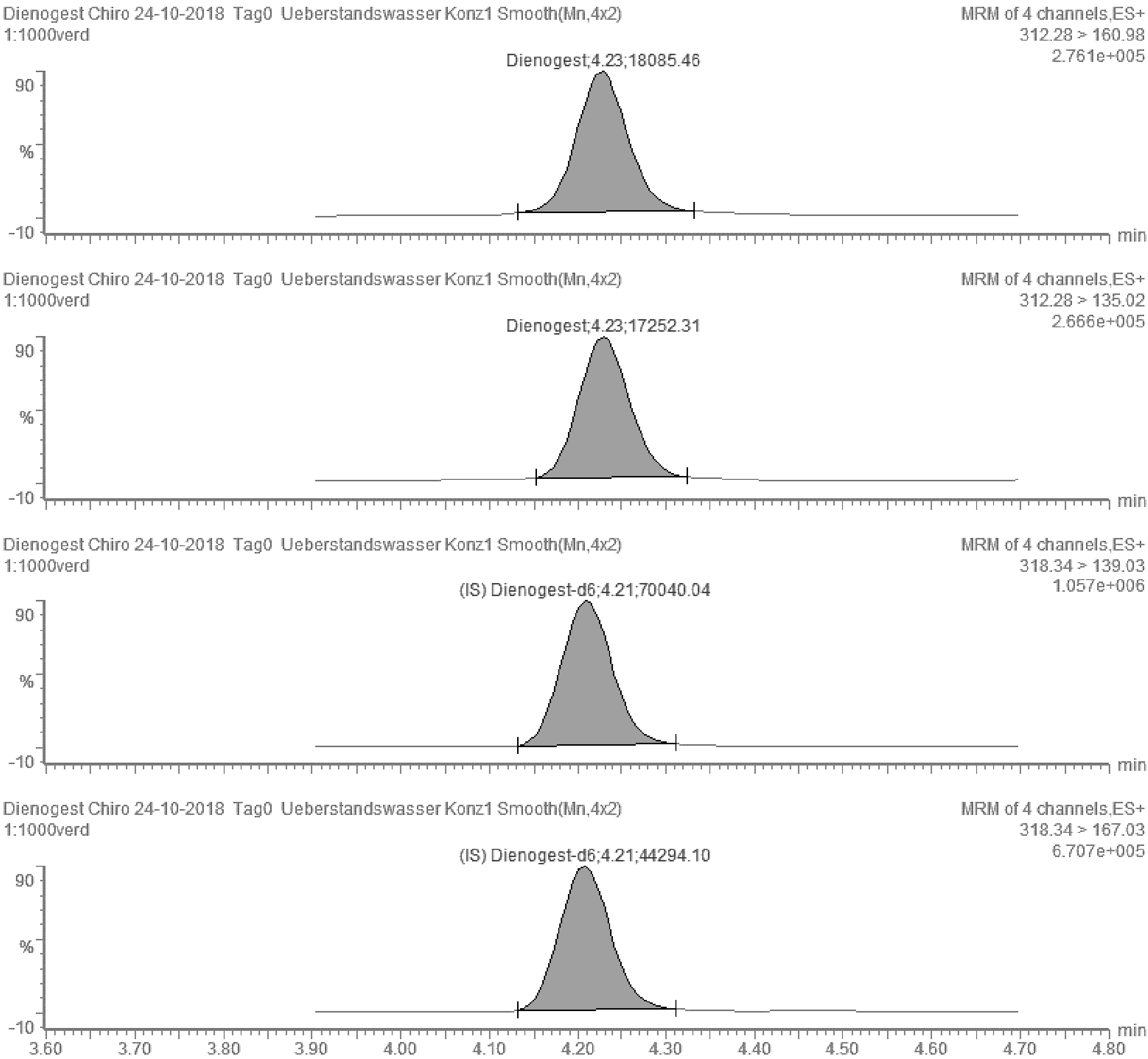
Source: Own graphic, Fraunhofer IME

Figure 8: Control sample, overlying water, sampling time: test start (October 24, 2018).



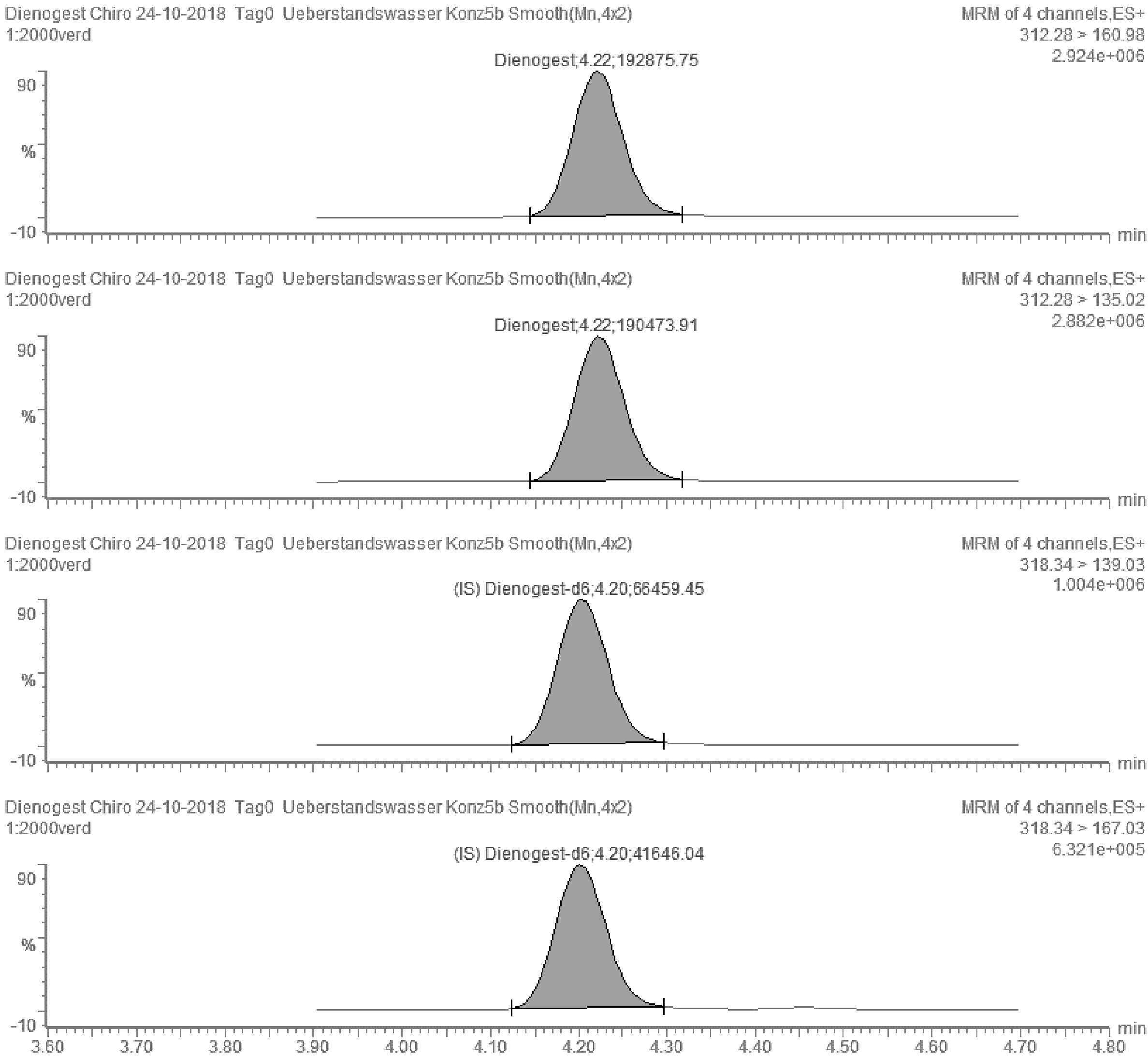
Source: Own graphic, Fraunhofer IME

Figure 9: Test sample concentration level 1, overlying water, test start (October 24, 2018).



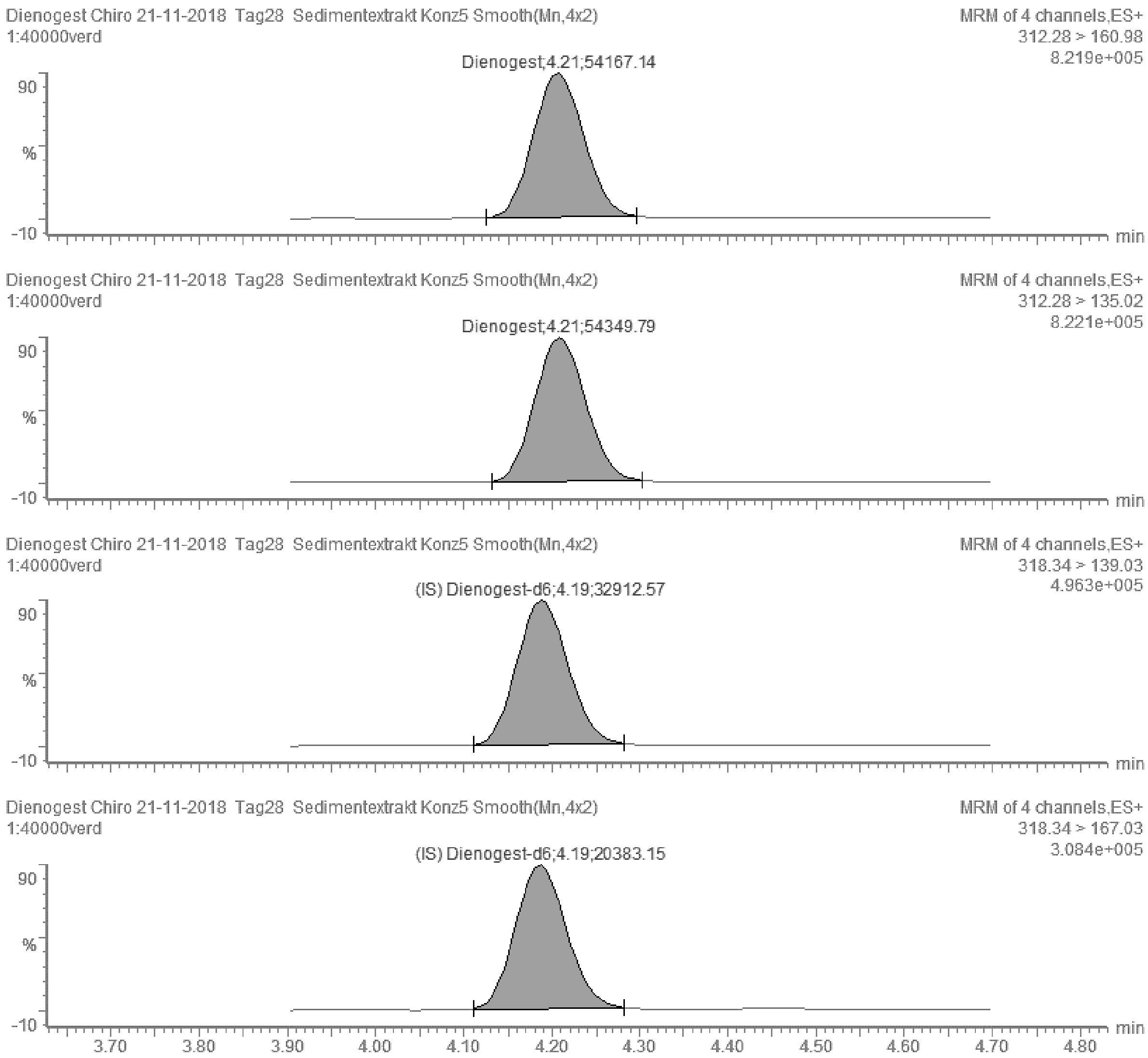
Source: Own graphic, Fraunhofer IME

Figure 10: Test sample concentration level 5, overlying water, test start (October 24, 2018).



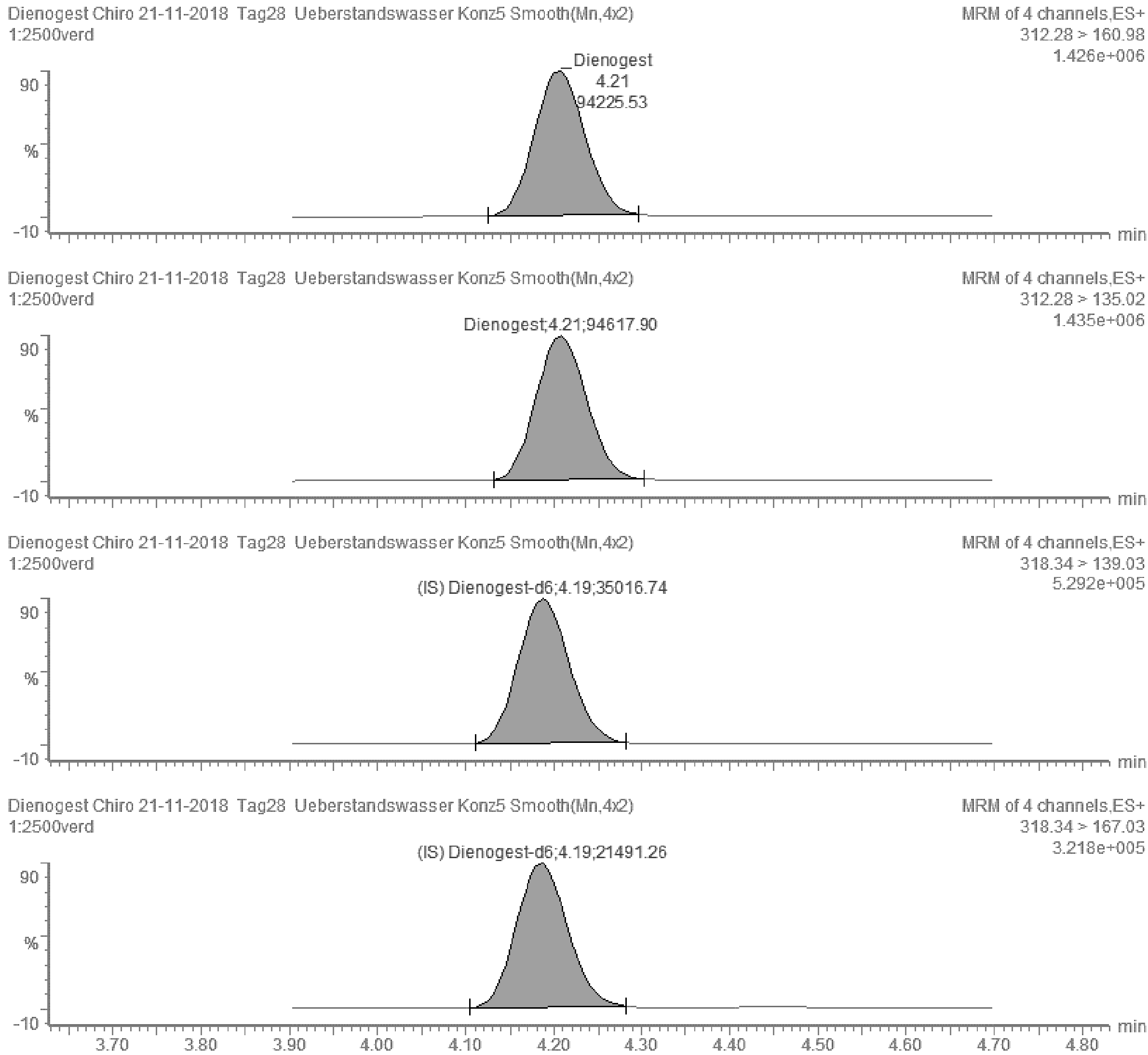
Source: Own graphic, Fraunhofer IME

Figure 11: Test sample concentration level 5, sediment, day 28 (November 21, 2018).



Source: Own graphic, Fraunhofer IME

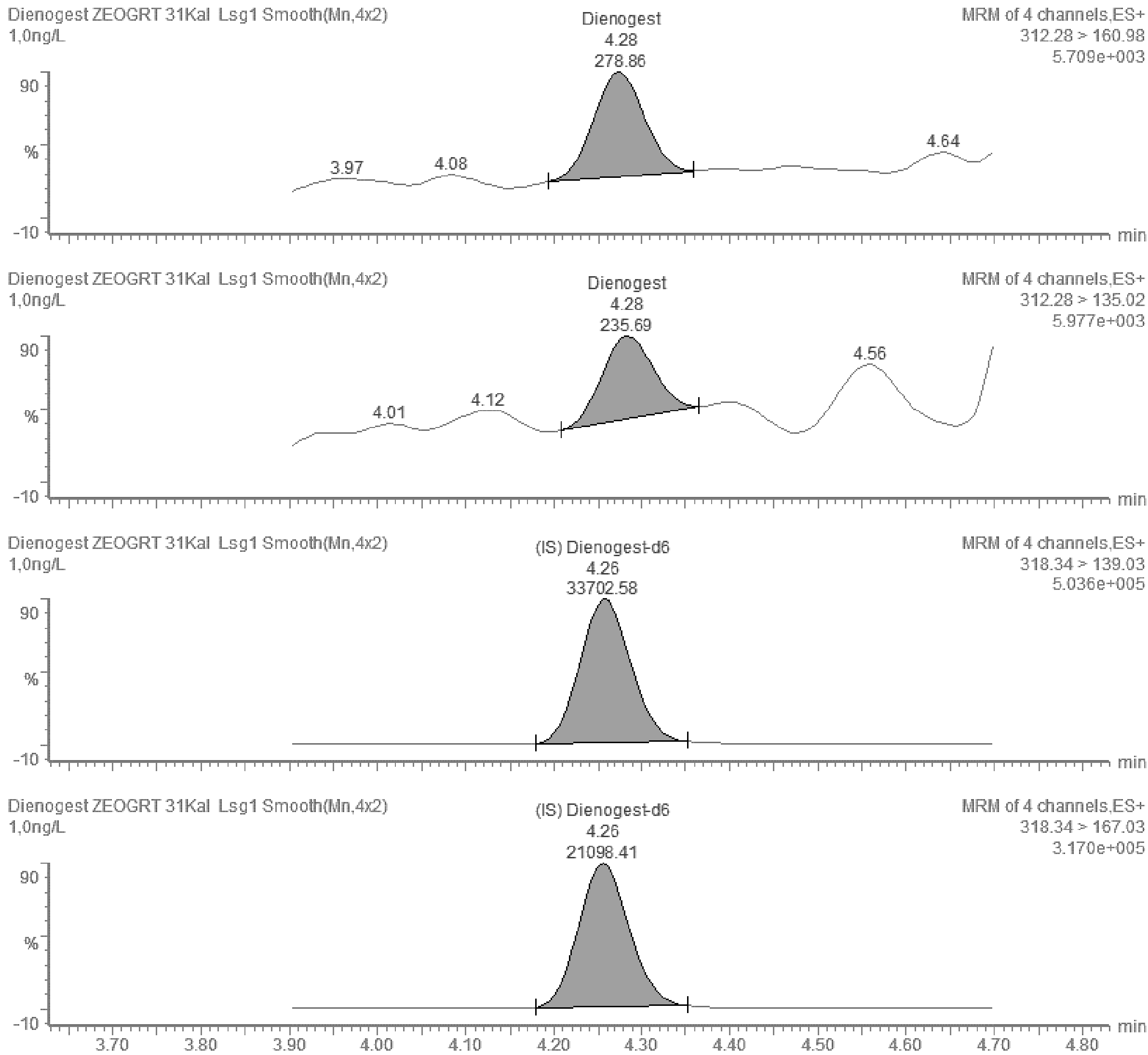
Figure 12: Test sample concentration level 5, overlying water, day 28 (November 21, 2018).



Source: Own graphic, Fraunhofer IME

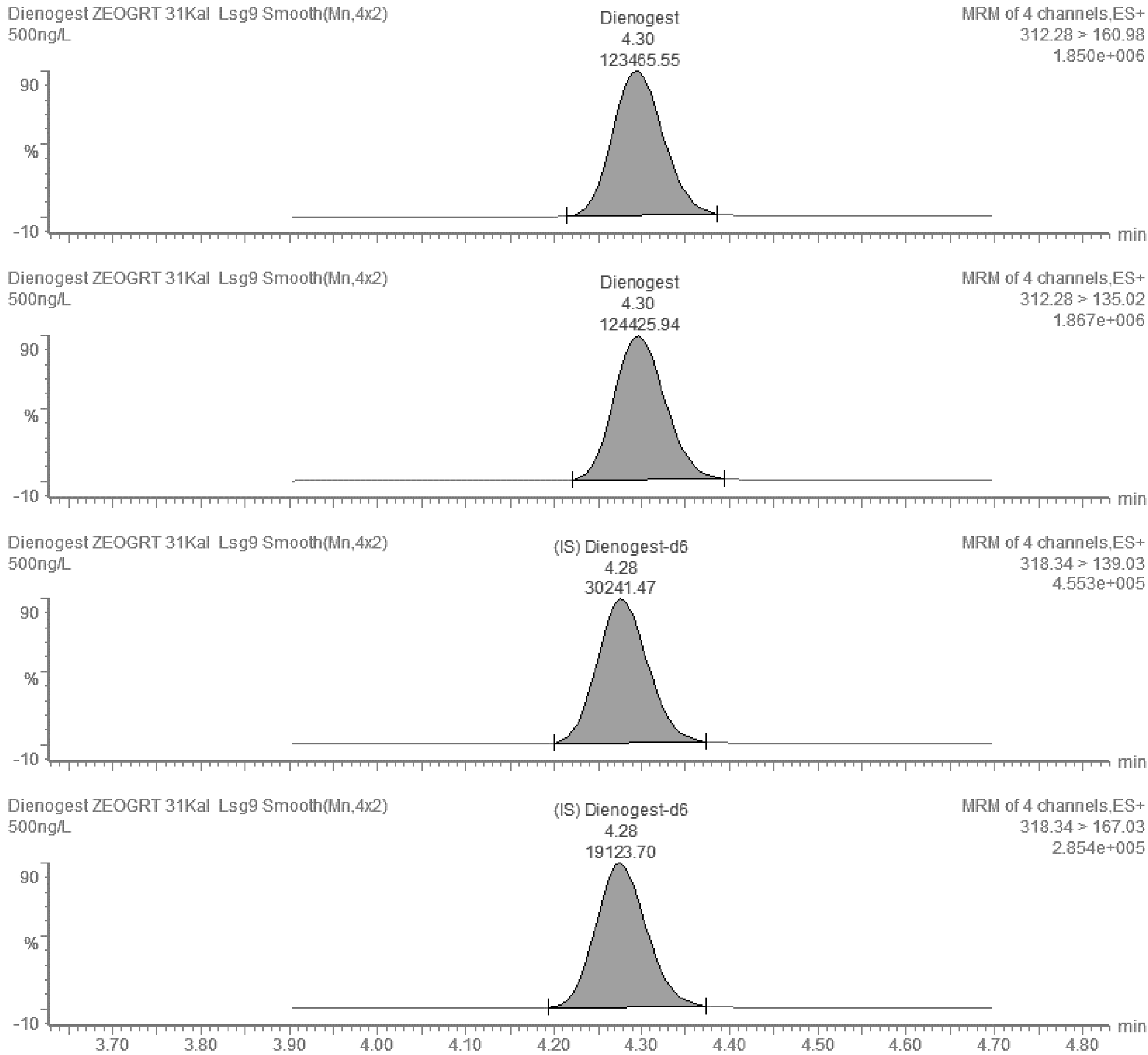
Zebrafish EOGRT

Figure 13: Calibration sample CS-1 measured on Nov 21, 2018; Dienogest conc.: 1.00 ng/L.



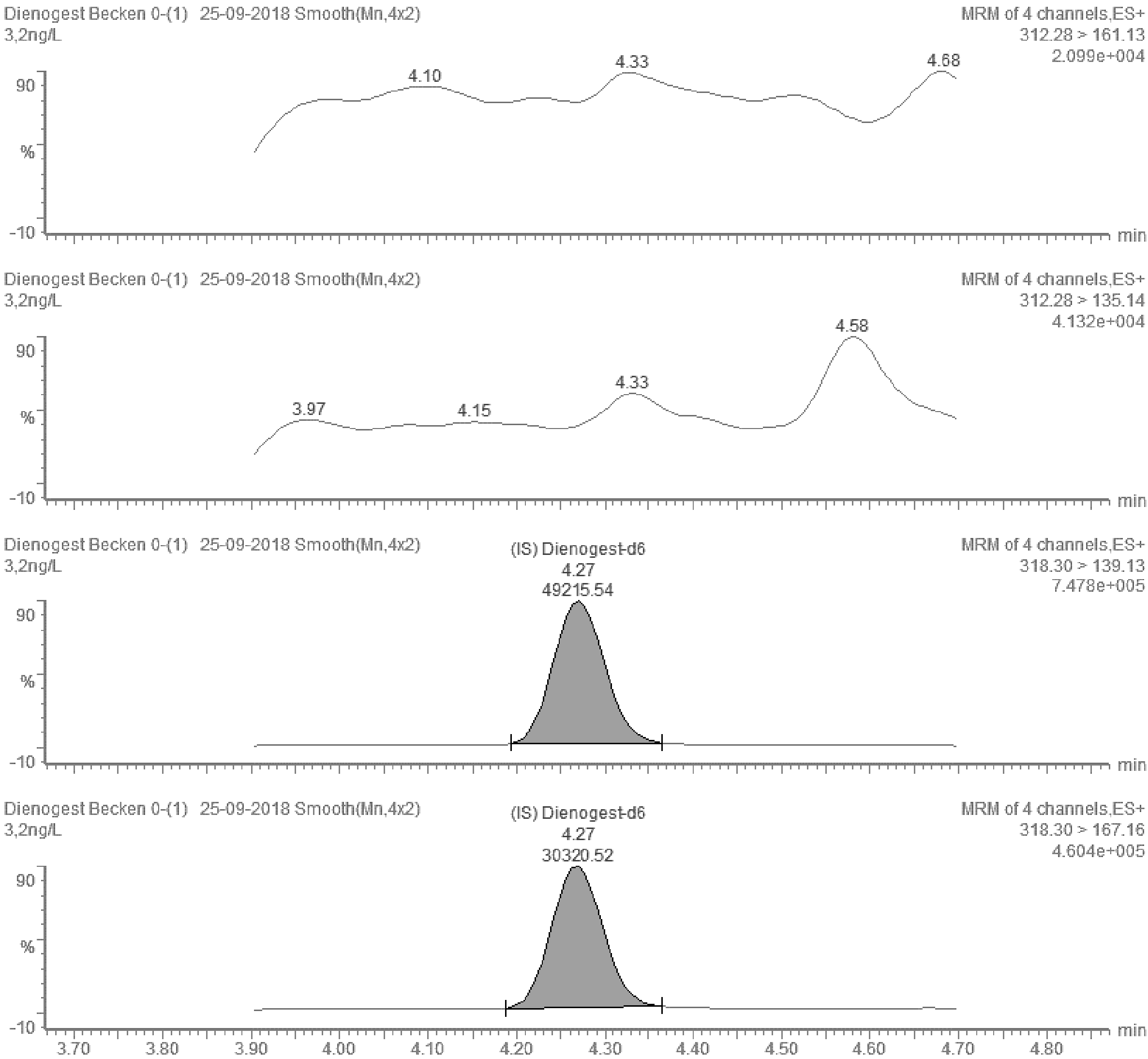
Source: Own graphic, Fraunhofer IME

Figure 14: Calibration sample CS-9 measured on Nov. 21, 2018; Dienogest conc.: 500 ng/L.



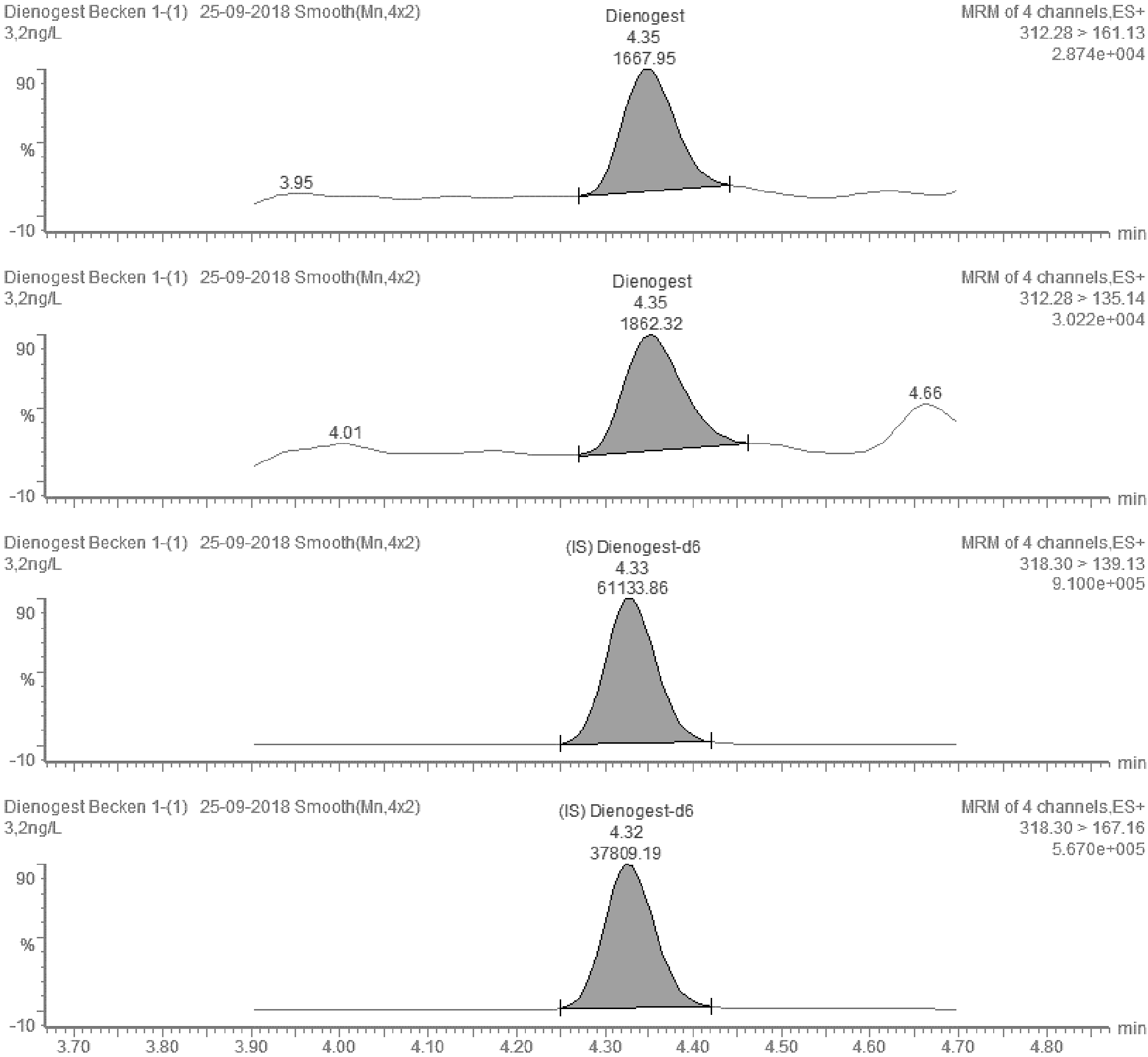
Source: Own graphic, Fraunhofer IME

Figure 15: Control sample, test medium, sampling time: test start (Sept. 25, 2018).



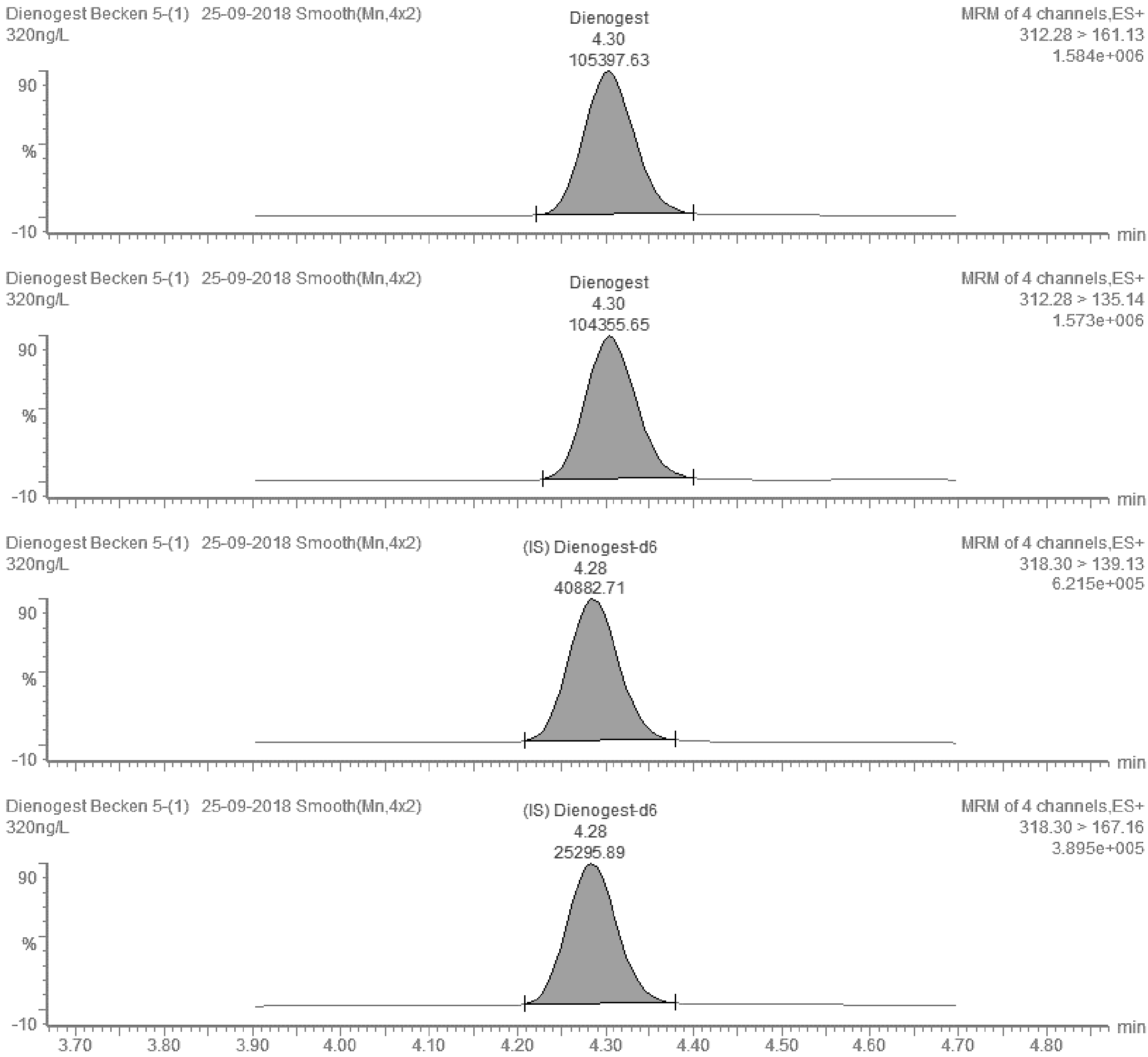
Source: Own graphic, Fraunhofer IME

Figure 16: Test media sample; nominal conc.: 3.20 ng/L; test start (Sept. 25, 2018).



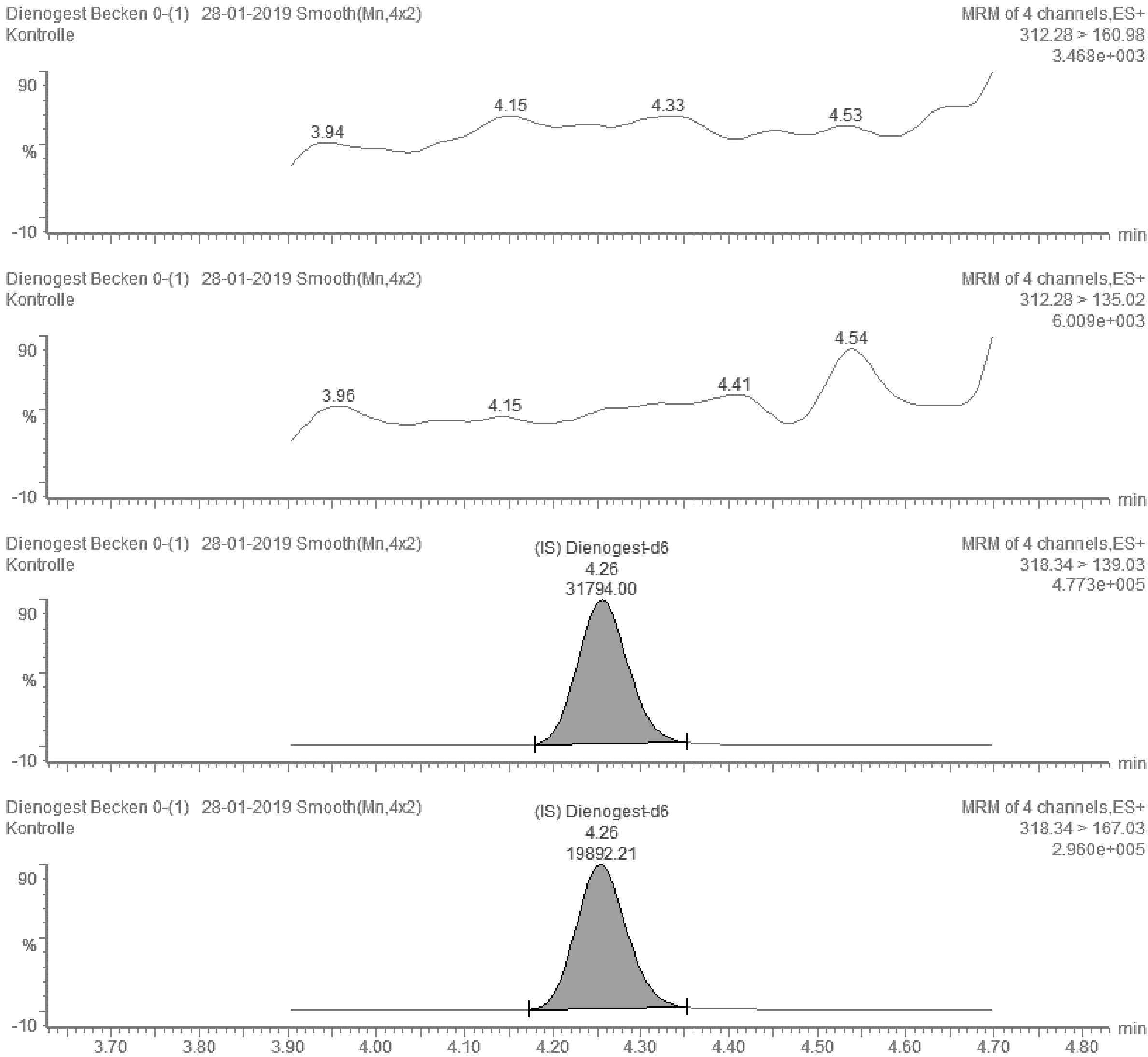
Source: Own graphic, Fraunhofer IME

Figure 17: Test media sample; nominal conc.: 320 ng/L; test start (Sept. 25, 2018).



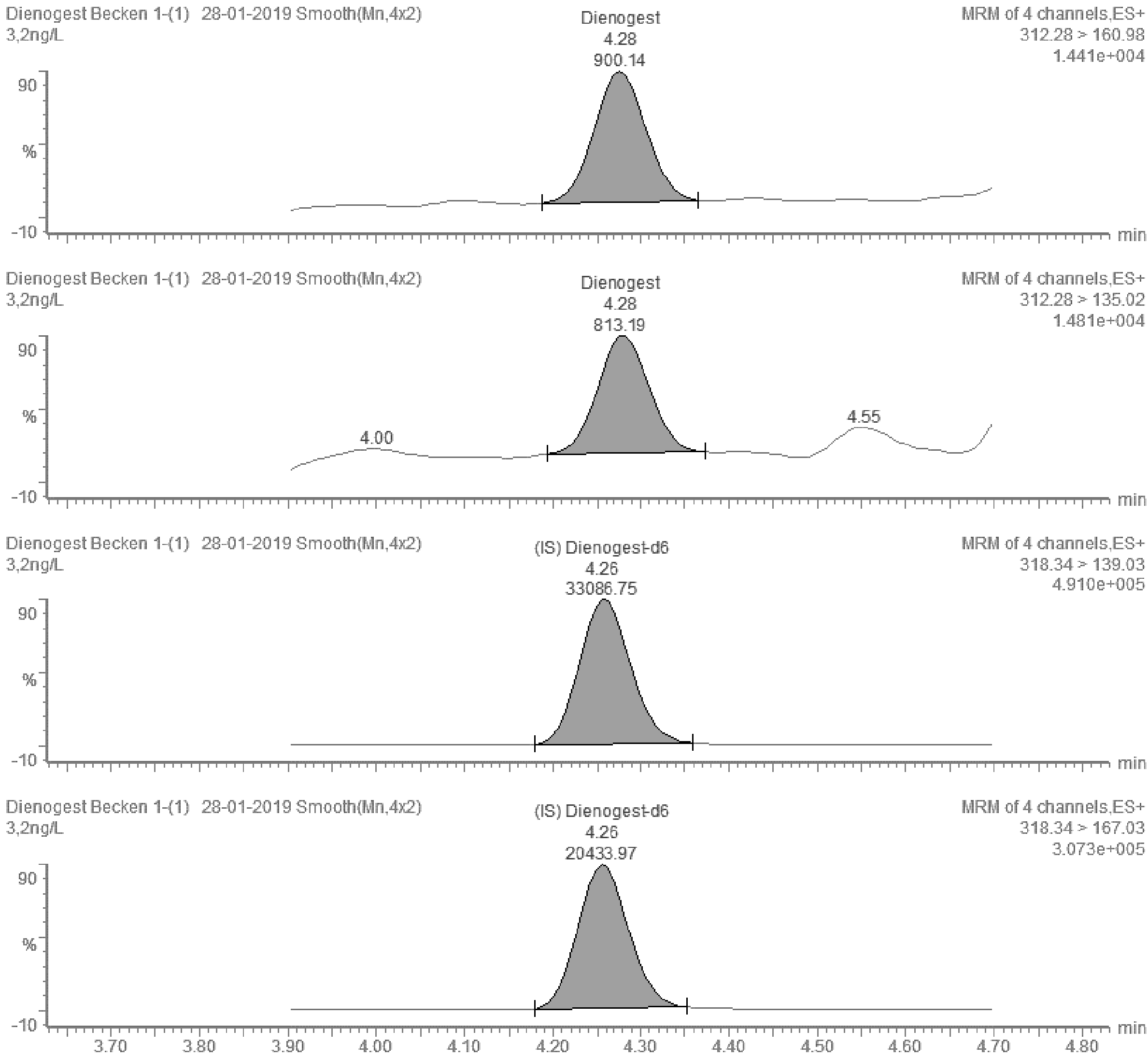
Source: Own graphic, Fraunhofer IME

Figure 18: Control sample, test medium, sampling time: test end (Jan. 28, 2019).



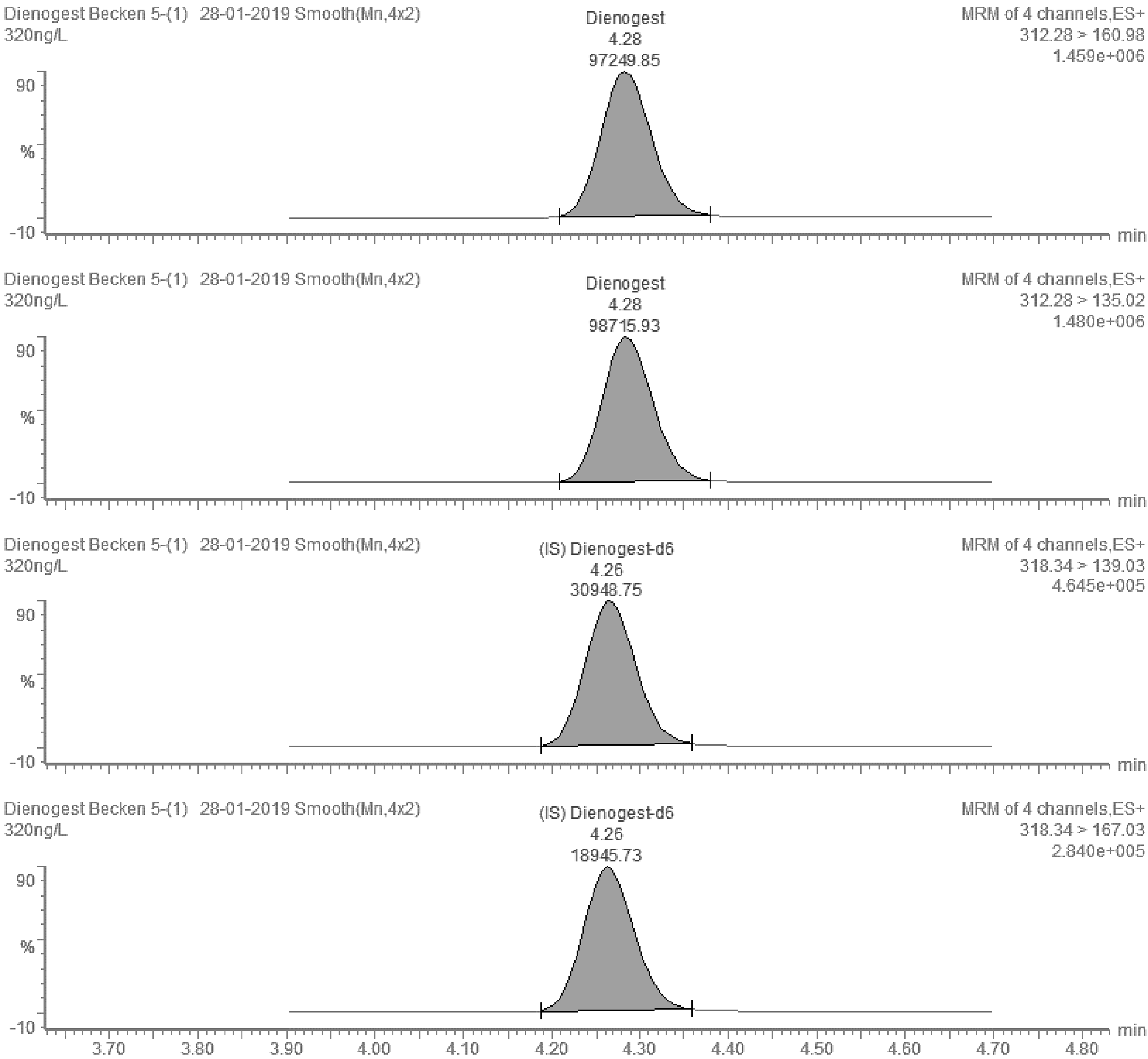
Source: Own graphic, Fraunhofer IME

Figure 19: Test media sample; nominal conc.: 3.20 ng/L; test end (Jan. 28, 2019).



Source: Own graphic, Fraunhofer IME

Figure 20: Test media sample; nominal conc.: 320 ng/L; test end (Jan. 28, 2019).



Source: Own graphic, Fraunhofer IME

B.1.8 Certificate of Analysis Dienogest

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Certificate of Analysis

Print

S1251 Dienogest

Research Area: Endocrinology & Hormones > Estrogen/progestogen Receptor > Dienogest

Name: **Dienogest**
Catalog Number: **S1251**
Batch Number: **S125103**

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(877) 796-6397
-- USA and Canada on

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+1-832-582-8590

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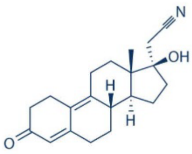
tech@selleckchem.cor
Please provide your (
Number in the email.
reply to all email inqu
one business day.

Website:
www.selleckchem.com

Physical and chemical properties

Molecular Formula:	$C_{20}H_{25}NO_2$		
Molecular Weight:	311.42		
CAS No.:	65928-58-7		
Stability:	3 years	-20°C	powder
	2 years	-80°C	in solvent

Molecular Structure:



Analytical data

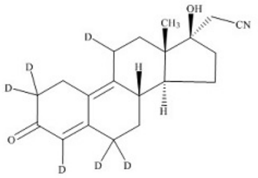
HPLC: 99.88% purity | NMR: Consistent with structure

B.1.9 Certificate of Analysis Dienogest-d6 (Internal Standard)

Medical Isotopes Inc.

Providing stable isotope chemicals and research compounds worldwide to universities, laboratories, hospitals, and government agencies.

MY CART

Product:	**Dienogest-d_6**	
Catalog Number:	D75937	
Unlabeled CAS Number:	65928-58-7	
Pricing:	1 mg = $590 (In Stock)	Add to Cart
Formula:	$C_{20}H_{19}NO_2D_6$	
Molecular Weight:	317.46	
Structure:		
Category:	Labeled Reference Standards	
MSDS:	Click here.	

B.2 Analytical report for Dexamethasone – Details of the method and results

B.2.1 Scope

The purpose of this analytical part of the study was to determine the analyte Dexamethasone in aqueous test medium (holding and dilution water) of a ZEOGRT study. As an additional study, a *Daphnia magna* reproduction test (according to OECD TG 211) was conducted at the laboratory of the German Federal Agency (UBA). Samples from that study were shipped to Fraunhofer IME and measured. The quantitative measurements were done by liquid chromatography coupled to a triple quadruple mass spectrometer; the MS was operated in the tandem mass spectrometry mode (LC-MS/MS).

The active substance Dexamethasone (CAS no. 50-02-2) was also used as the analytical standard; isotopically labelled Dexamethasone-d4 (Dexamethasone-4,6α,21,21-d4) was used as internal standard (IS).

B.2.2 Chemicals, reagents and analytical equipment

- Analytical standard Dexamethasone, Batch: BCBW7684, Purity (HPLC): 100 area%, Recommended retest date: FEB 2023, Product no.: D1756 (Sigma-Aldrich), according to the respective Certificate of Analysis, see data sheet in chapter B.2.8.
- Internal Standard, Dexamethasone-d4, Lot no.: X-473, Chemical purity: 98.2%, Expiration: re-analyzed after three years, Product no.: D-5559 (CDN Isotopes), see data sheet in chapter B.2.9
- IS solution, solution of the internal standard Dexamethasone-d4 in methanol, conc.: 200 µg/L
- Methanol, 'Methanol for LC-MS', Article No. 1428 (Th. Geyer)
- Ammonium acetate, 'Optima LC-MS', Article No. 11317490 (Fisher Scientific)
- Formic acid, 'Optima LC-MS', Article No. 10596814 (Fisher Scientific)
- Purified water, produced with purification system Purelab® Ultra (ELGA LabWater)
- Solvent mixture 1: Mixture of 100 mL purified water and 20 mL methanol
- Analytical balance XPE 205 DR (Mettler Toledo)
- Piston operated pipette, 'research 5000', variable volume selection (Eppendorf)
- 12 mL screw cap vials, 15 mm thread, 66 x 19 mm, clear glass; equipped with15 mm screw caps with Teflon coated sealing disks (WiCom)
- Common laboratory equipment (volumetric flasks, glass beakers, Pasteur pipettes etc.)
- Screw top vials, approx. 2.0 mL capacity, clear glass; screw caps with Teflon coated sealing disks (WiCom)
- MicromanTM pipettes (positive displacement), M25, M50 and M250 (Fisher Scientific)

B.2.3 Sampling and sample processing

Sampling

The samplings were performed by the staff of the department of environmental and food analysis using piston operated pipettes. To prevent degradation of the analyte and to minimize wall effects the aqueous samples were stabilized by adding methanol. Therefore, aliquots of accurately 1.00 mL methanol were filled into each 12 mL screw cap vials prior to start of the sampling procedure. Subsequent 5.00 mL of the aqueous test media were taken out of the test vessels and were pipetted into the screw top vials as well; the volume mix water/methanol was therefore 5+1 (v/v). After vigorously mixing by hand the samples were handed over to the chemical laboratory for immediate analysis; if this was not possible the samples were stored in a freezer at a temperature of ≤ -18°C until analysis.

42 samples originated from an external study with Daphnia. 5 mL of test medium had been stabilized with 1.0 mL of methanol. Samples were kept frozen until further processing and analysis.

Sample processing

For direct LC-MS/MS measuring all samples were thawed and allowed to equilibrate to room temperature. Aliquots of 1.20 mL of the aqueous samples (water/methanol mixtures) were then filled into 2 mL screw top vials; afterwards 50.0 µL of the IS solution IS-IM-1a were added and the vials were mixed by hand. If necessary, aliquots of the aqueous samples (water/methanol mixtures) were diluted with solvent mixture 1 to generate analyte concentrations which corresponds to concentration range of the basic calibration (dilution factor (D_F) of undiluted samples = 1). Finally, 100 µL of the processed sample were injected into the LC-MS/MS system, the measuring method is described in detail in chapter B.2.4. The remaining water/methanol mixtures were stored in a freezer at a temperature of ≤ -18 °C to allow a second measurement.

B.2.4 LC-MS/MS measurement

The quantitative determination of Dexamethasone was carried out by liquid chromatography and tandem mass spectrometry detection (LC-MS/MS) using positive electrospray ionization (ESI+). The measurement conditions and instrument settings are summarized below.

LC-MS/MS system

HPLC system:	Waters 2695
Mass spectrometer:	Waters LC-MS/MS Quattro Micro (triple quadruple system)
Software:	Waters MassLynx Ver. 4.1
Quantitation software:	Waters QuanLynx Ver. 4.1

LC parameters

Column:	Phenomenex Gemini C18; 5 µm, 150 mm x 3 mm
Guard Column:	Phenomenex Gemini C18; 5 µm, 4 mm x 3 mm
Column temperature:	30°C
Injection volume:	100 µL
Flow rate:	0.50 mL/min
Mobile phase A:	1000 mL methanol + 2mL 1 M ammonium acetate solution
Mobile phase B:	900 mL purified water + 100 mL methanol + 2 mL 1 M ammonium acetate solution
Mobile phase C:	1000 mL methanol + 2mL 1 M formic acid

Gradient program

Time [min]	Solvent A [%]	Solvent B [%]	Solvent C [%]
0.00	45	50	5.0
1.00	45	50	5.0
3.00	95	0	5.0
5.00	95	0	5.0
5.20	45	50	5.0
8.00	45	50	5.0

MS method (measurement conditions and instrument setting)

Type:	MRM
Ion mode:	ESI+
Span:	0.2 Da
Solvent delay 1:	0.10 – 4.50 min
Solvent delay 2:	7.00 – 8.00 min
End time:	8.00 min
Collision gas:	Argon

Usage of MRM transition

Substance (indication)	Retention time [min]	Precursor ion [m/z]	Product ion [m/z]	Cone voltage [V]	Collision energy [eV]	Dwell time [s]
Analyte, quantitation ion	5.3	393.23	373.17	15	10	0.100
Analyte, qualifier ion		393.23	355.17	15	12	0.100
IS, quantitation ion	5.3	397.24	377.17	15	10	0.100
IS, qualifier ion		397.24	359.17	15	12	0.100

MS parameters

Source settings		Analyzer settings	
Capillary:	1.00 kV	LM 1 resolution:	13.7
Cone:	15.00 V	HM 1 resolution:	13.7
Extractor:	2.00 V	Ion energy 1:	0.5
RF Lens:	0.1 V	MS Mode Entrance:	1
Source Temperature [°C]:	120°C	MS Mode Exit:	1
Desolvation Temperature:	350°C	LM 2 resolution:	13.7
Cone Gas Flow:	100 L/h	HM 2 resolution:	13.7
Desolvation Gas Flow:	600 L/h	Ion energy 2:	1.0
		Multiplier (V):	650

B.2.5 Calibration, quantification and calculation of the analytical results

Stock and intermediate solutions of the analyte and the internal standard

The stock solutions of analytical standard Dexamethasone and the internal standard (IS) Dexamethasone-d4 were prepared by exactly weighing the standard compounds directly into separate volumetric flasks and by subsequent filling up to the ring mark with methanol, see Table 11. Solution S-1a was prepared in ACN.

Table 11: Preparation of stock solutions of the analyte and the internal standard.

Stock solution	Compound	Date of preparation	Weighed amount	Purity	Volumetric flask, nominal volume	Resulting concentration
S-1a	Dexamethasone	Aug. 29, 2019	20.40 mg	100%	20 mL	1.020 g/L
S-2a	Dexamethasone	Sept. 19, 2019	10.60 mg	100%	20 mL	0.530 g/L
IS-1a	Dexamethasone-d4	Sept. 18, 2019	5 mg *	98.2%	5 mL	0.986 g/L
S-2b	Dexamethasone	Nov. 29, 2019	12.01 mg	100%	20 mL	0.601 g/L
S-2c	Dexamethasone	Sept. 19, 2019	12.00 mg	100%	20 mL	0.600 g/L

*Total amount delivered

For the preparation of the calibration solutions intermediate solutions (IM) were prepared by pipetting aliquots of the analyte stock S-1a or the IM solution IM-1a into additional volumetric flasks and filling up to the mark with methanol, the resulting concentrations and the dilution scheme are given in Table 12.

Table 12: Preparation of analyte intermediate solution (IM set 1).

Analyte intermediate (IM) solution	Date of preparation	Used solution	Used volume	Volumetric flask, nominal volume	Resulting concentration
IM-1a	Sept 19, 2019	S-1a	49.02 µL	10 mL	5.00 mg/L
IM-2a	Sept 19, 2019	IM-1a	400.0 µL	10 mL	200.0 µg/L

For spiking the test media samples during sample processing (cp. chapter B.2.3) and preparation of the calibration solutions an IM solution of the IS was prepared by dilution with methanol, the resulting concentration and the dilution scheme is given in Table 13.

Table 13: Preparation of internal standard spiking solution.

IS intermediate (IM) solution	Date of preparation	Used solution	Used volume	Volumetric flask, nominal volume	Resulting concentration
IS-IM-1a	Sept 19, 2019	IS-1a	10.14 µL	50 mL	0.200 mg/L

For the preparation of the QC standards an additional set of analyte IM solutions was prepared in methanol based on the analyte stock S-2a, 2b or 2c for later time points; the solutions, the dilution scheme and the resulting concentrations are given in Table 14.

Table 14: Preparation of analyte intermediate solution (IM set 2).

Analyte intermediate (IM) solution	Date of preparation	Used solution	Used volume	Volumetric flask, nominal volume	Resulting concentration
IM-3a	Sept 19, 2019	S-2a	37.74 µL	25 mL	2.00 mg/L
IM-4a	Sept 19, 2019	IM-3a	250.0 µL	20 mL	50.0 µg/L
IM-3b	Nov. 29, 2019	S-2b	33.31 µL	10 mL	2.00 mg/L
IM-4b	Nov. 29, 2019	IM-3b	250.0 µL	20 mL	50.0 µg/L
IM-3c	Feb. 17, 2020	S-2c	33.33 µL	25 mL	2.00 mg/L
IM-4c	Feb. 17, 2020	IM-3c	250.0 µL	20 mL	50.0 µg/L

Preparation of the calibration standards and the matrix calibration samples

Due to the expected higher stability of the analyte in an organic solvent the 'calibration standards' (calibration solutions) were prepared and stored in pure methanol.

Eight calibration standards (C-1a, C-2a, ...) were prepared on September 19, 2019 in the concentration range from 0.400 to 250 µg/L by diluting the intermediate solutions IM-1a or IM-2a in volumetric flasks with methanol; see pipetting plan in Figure 16 (microman pipettes were used for this dilution step).

The 'basic calibration samples' (CS-1, CS-2, ...) were prepared afterwards by mixing 200 µL of the calibration standards and 50 µL of the IS solution IS-IM-1a with 1000 µL purified water in 2 mL screw top vials; the volume mix water/methanol was therefore = 5+1 (v/v), this was the same solvent composition as it existed at the end of sample processing, cp. chapter B.2.3. The calibration samples were measured as described in chapter B.2.4.

All prepared calibration standards were stored at approximately 4°C in a refrigerator.

Table 15: Preparation o the calibration standards and the calibration samples, analyte Dexamethason

No. of the calibration solution	Volume solution IM-1a	Volume solution IM-2a	Volumetric flask, nominal volume	Analyte concentration, calibration solution	No. of the calibration sample	Analyte concentration, calibration sample *
C-1a	-	20.00 µL	10 mL	0.400 µg/L	CS-1	0.080 µg/L
C-2a	-	125.0 µL	10 mL	2.50 µg/L	CS-2	0.500 µg/L
C-3a	-	250.0 µL	10 mL	5.00 µg/L	CS-3	1.00 µg/L
C-4a	-	500.0 µL	10 mL	10.0 µg/L	CS-4	2.00 µg/L
C-5a	50.00 µL	-	10 mL	25.0 µg/L	CS-5	5.00 µg/L
C-6a	100.0 µL	-	10 mL	50.0 µg/L	CS-6	10.0 µg/L
C-7a	250.0 µL	-	10 mL	125 µg/L	CS-7	25.0 µg/L
C-8a	500.0 µL	-	10 mL	250 µg/L	CS-8	50.0 µg/L

*) Remark: The concentrations of the final calibration samples were related to the analyte amounts in the methanol/water mixture. They are lower than the actual concentrations as the added volumes of the calibration standards (CS-1, CS-2, …and the IS solution are not considered. These concentrations correspond to the concentrations of test samples to be analyzed by this method. For analysis of (aqueous) test samples equal amounts of the solvent used in the calibration standards are added to the test samples. Thus, the test samples are treated in the same manner as the calibration samples and contained water and solvent at same concentrations (volume mixture methanol/water = 1+5, cp. chapter B.2.3).

This procedure was repeated on every measuring day and new matrix calibration functions were recorded or old calibrations were checked using QC samples.

Basic calibration of the LC-MS/MS system and creating the calibration function

The LC-MS/MS system was calibrated for the analysis of the aqueous test samples by preparation and measuring of the prepared calibration samples (cp. previous chapters). Afterwards the chromatographic raw data were processed (integrated) using the Waters Quan-Lynx software. Subsequent the calibration functions were set up by the 'internal standard method' plotting the peak area ratios (PAR = integrated peak area analyte / integrated peak area IS) against the used analyte concentrations. With the received calibration data a linear regression calculation was performed.

Quantification and calculation of the analytical results

The LC-MS/MS quantification data were generated by processing the chromatographic raw data of the measured samples and by subsequent calculation of the quantification results ($C_{LC\text{-}MS/MS}$) using the respective matrix calibration function.

As the aqueous test samples (water) and the calibration samples were pre-treated (diluted) in the same way and were analyzed by direct injection into the LC-MS/MS system, the concentrations of the analyte in the aqueous test samples (C_W) were quantified directly from the relevant calibration function ($C_{LC\text{-}MS/MS} = C_W$).

Quality control

Two quality control (QC) standards were used for the verification of the basic calibration. The QC standard solutions were prepared analogous to calibration standards, but were based on separate weights of the analytical standard, Table 11 (solutions S-1b).

The QC standard solutions QC-1a and QC-2a were prepared on September 19, 2019 in concentrations of 10.0 µg/L and 200 µg/L by diluting the IM-3a solution (2.00 mg Dexamethasone/L) in separate volumetric flasks with methanol; see pipetting plan in Table 16. On the day of measurement, the QC samples QC-S-1a and QC-S-2a were prepared by mixing 200 µL of the QC standard solutions with 50.0 µL of IS solution IS-IM-1a and 1000 µL purified water in screw top vials (volume mixture water/methanol = 5+1 (v+v). The analyte concentration in the QC samples were thus 2.00 µg/L and 40.0 µg/L, the QC samples were measured in turn as described in chapter B.2.4. Fresh QC standards and samples were prepared November 29, 2019 and February 17, 2020 respectively. The prepared QC standard solutions were stored in a refrigerator as well.

Table 16: Preparation of quality control (QC) standards and QC samples.

No. of the QC standard	Volume solution IM-3a	Volumetric flask, nominal volume	Analyte concentration, QC solutions	No. of the QC sample	Analyte concentration, QC samples *)
QC-1a (b or c)	50.0 µL	10 mL	10.0 µg/L	QC-S-1a (b or c)	2.00 µg/L
QC-2a (b or c)	1000 µL	10 mL	200 µg/L	QC S-2a (b or c)	40.0 µg/L

*) The comment to Table 52 also applies to the analyte concentrations of the QC samples

The measurement intervals of the QC standards were every twentieth sample, but at least once a measurement day.

B.2.6 Results

Matrix calibration function

The basic calibration function (response type: internal standard) used for the quantification of Dexamethasone in the measured aqueous test medium samples was measured on October 15, 2019 and is shown in Figure Figure 21; the calibration function was calculated by linear regression analysis using the Waters QuanLynx software to:

Function: PAR = 0.114596 • C_{Cal} - 0.00172 r^2 = 0.999682

PAR = Peak area ratio

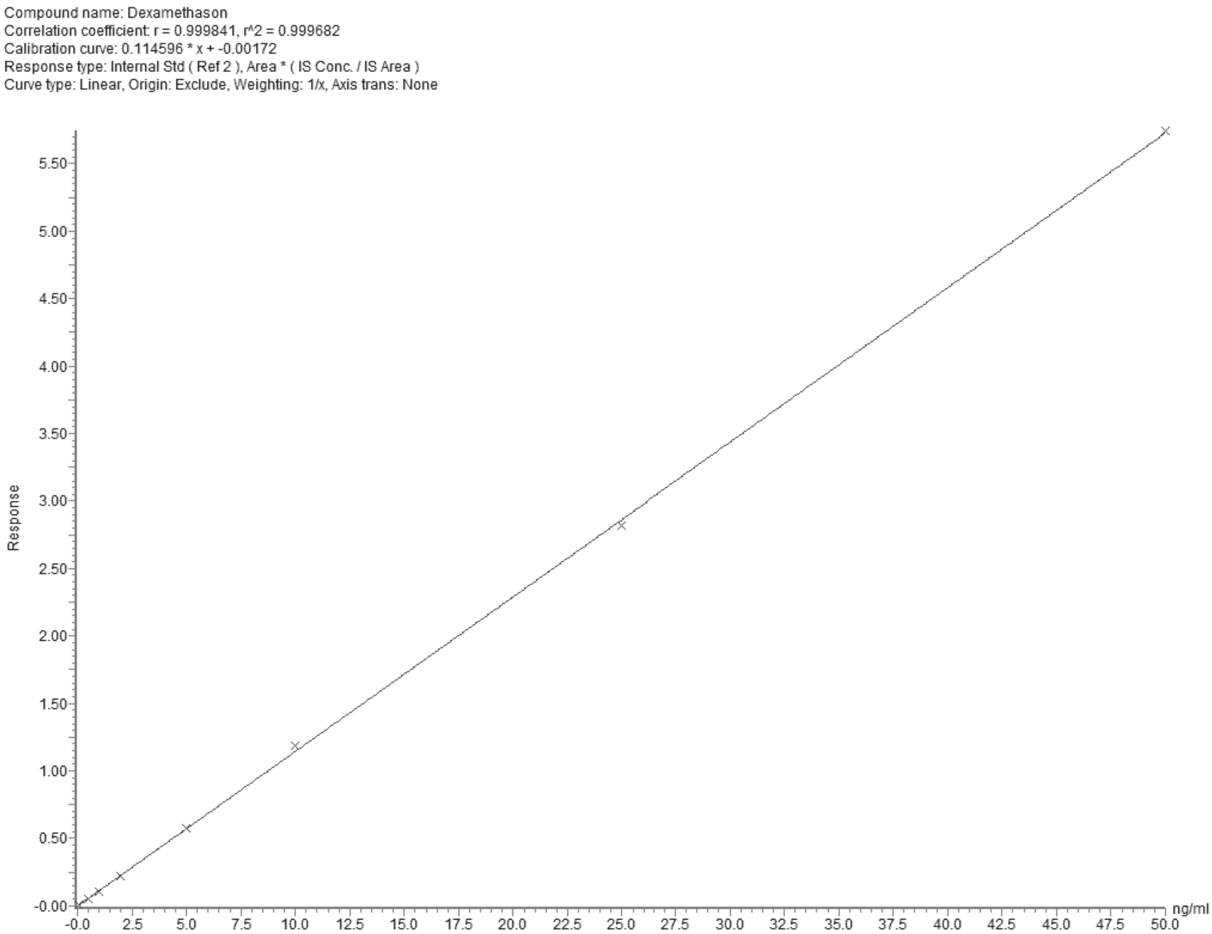
C_{Cal} = Analyte concentration of the calibration solutions

r^2 = Coefficient of determination

Linearity

Using the linear regression model, the coefficient of determination for Dexamethasone was calculated to 0.999682. As the calculated r^2-values were very close to 1, the linearity of the calibration functions was accepted.

Figure 21: Basic calibration function of Dexamethasone as measured October 16, 2019.



Source: Own graphic, Fraunhofer IME

Results of the analysed samples

ZEOGRT: The Dexamethason concentrations in the aqueous phase were determined in all replicates of all treatment levels and controls at test start. Thereafter, 2 replicates of each treatment level and control were sampled and analysed for each sampling time point. The analytical results are listed in Table 17; in addition to samples information, the quantification data, the measured analyte concentrations in water (CW) and the calculated 'Percent of nominal' values were inserted into the table.

Table 17: Analysed Dexamethasone concentrations (C_W) and corresponding recovery values (mass transition m/z 393.23 → m/z 373.17)

Sampling, date and time (2019)	Treat-ment	Nominal Dexa-metha-sone conc.	Test vessel	Dilution factor, DF	LC-MS/MS quantific. data, CLC-MS/MS	Measured analyte conc., CW	Percent of nominal
October 17, 2019 08:15	Control	0.00 µg/L	1	1	<LOQ*	<LOQ	-
			2	1	<LOQ*	<LOQ	-
			3	1	<LOQ*	<LOQ	-
			4	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	1	1	0.270 µg/L	0.270 µg/L	84.4%
			2	1	0.305 µg/L	0.305 µg/L	95.3%
			3	1	0.281 µg/L	0.281 µg/L	87.8%
			4	1	0.281 µg/L	0.281 µg/L	87.8%
	Conc. 2	1.00 µg/L	1	1	0.760 µg/L	0.760 µg/L	76.0%
			2	1	0.793 µg/L	0.793 µg/L	79.3%
			3	1	0.840 µg/L	0.840 µg/L	84.0%
			4	1	0.815 µg/L	0.815 µg/L	81.5%
	Conc. 3	3.20 µg/L	1	1	2.62 µg/L	2.62 µg/L	82.0%
			2	1	2.51 µg/L	2.51 µg/L	78.5%
			3	1	2.66 µg/L	2.66 µg/L	83.0%
			4	1	2.54 µg/L	2.54 µg/L	79.4%
	Conc. 4	10.0 µg/L	1	1	7.71 µg/L	7.71 µg/L	77.1%
			2	1	8.20 µg/L	8.20 µg/L	82.0%
			3	1	8.80 µg/L	8.80 µg/L	88.0%
			4	1	8.94 µg/L	8.94 µg/L	89.4%
	Conc. 5	32.0 µg/L	1	1	29.1 µg/L	29.1 µg/L	90.8%
			2	1	29.4 µg/L	29.4 µg/L	91.9%
			3	1	26.1 µg/L	26.1 µg/L	81.7%
			4	1	26.4 µg/L	26.4 µg/L	82.5%
	Conc. 6	100 µg/L	1	4	18.8 µg/L	75.2 µg/L	75.2%
			2	4	18.7 µg/L	74.6 µg/L	74.6%
			3	4	19.6 µg/L	78.3 µg/L	78.3%
			4	4	19.7 µg/L	78.8 µg/L	78.8%

Sampling, date and time (2019)	Treat-ment	Nominal Dexa-metha-sone conc.	Test vessel	Dilution factor, DF	LC-MS/MS quantific. data, CLC-MS/MS	Measured analyte conc., CW	Percent of nominal
October 18, 2019 08:15	Conc. 6	100 µg/L	1	4	23.3 µg/L	93.2 µg/L	93.2%
			2	4	23.4 µg/L	93.8 µg/L	93.8%
			3	4	24.2 µg/L	96.6 µg/L	96.6%
			4	4	24.8 µg/L	99.4 µg/L	99.4%
October 23, 2019	Control	0.00 µg/L	1	1	<LOQ*	<LOQ	-
			3	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	1	1	0.324 µg/L	0.324 µg/L	101%
			3	1	0.344 µg/L	0.344 µg/L	108%
	Conc. 2	1.00 µg/L	1	1	0.928 µg/L	0.928 µg/L	92.8%
			3	1	0.928 µg/L	0.928 µg/L	92.8%
	Conc. 3	3.20 µg/L	1	1	2.76 µg/L	2.76 µg/L	86.1%
			3	1	2.97 µg/L	2.97 µg/L	92.8%
	Conc. 4	10.0 µg/L	1	1	9.63 µg/L	9.63 µg/L	96.3%
			3	1	11.5 µg/L	11.5 µg/L	115%
	Conc. 5	32.0 µg/L	1	1	36.6 µg/L	36.6 µg/L	114%
			3	1	34.2 µg/L	34.2 µg/L	107%
	Conc. 6	100 µg/L	1	4	25.9 µg/L	103 µg/L	103%
			3	4	27.1 µg/L	109 µg/L	109%
October 31, 2019	Control	0.00 µg/L	2	1	<LOQ*	<LOQ	-
			4	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	2	1	0.307 µg/L	0.307 µg/L	95.9%
			4	1	0.334 µg/L	0.334 µg/L	104%
	Conc. 2	1.00 µg/L	2	1	1.02 µg/L	1.02 µg/L	102%
			4	1	0.975 µg/L	0.975 µg/L	97.5%
	Conc. 3	3.20 µg/L	2	1	3.201 µg/L	3.20 µg/L	100%
			4	1	3.404 µg/L	3.40 µg/L	106%
	Conc. 4	10.0 µg/L	2	1	10.9 µg/L	10.9 µg/L	109%
			4	1	12.6 µg/L	12.6 µg/L	126%
	Conc. 5	32.0 µg/L	2	1	39.0 µg/L	39.0 µg/L	122%
			4	1	36.3 µg/L	36.3 µg/L	113%

Sampling, date and time (2019)	Treat-ment	Nominal Dexa-metha-sone conc.	Test vessel	Dilution factor, DF	LC-MS/MS quantific. data, CLC-MS/MS	Measured analyte conc., CW	Percent of nominal
	Conc. 6	100 µg/L	2	4	28.0 µg/L	112 µg/L	112%
			4	4	27.1 µg/L	108 µg/L	108%
November 05, 2019	Control	0.00 µg/L	1	1	<LOQ*	<LOQ	-
			3	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	1	1	0.344 µg/L	0.344 µg/L	108%
			3	1	0.329 µg/L	0.329 µg/L	103%
	Conc. 2	1.00 µg/L	1	1	0.986 µg/L	0.986 µg/L	98.6%
			3	1	1.01 µg/L	1.01 µg/L	101%
	Conc. 3	3.20 µg/L	1	1	3.34 µg/L	3.34 µg/L	104%
			3	1	2.99 µg/L	2.99 µg/L	93.4%
	Conc. 4	10.0 µg/L	1	1	10.9 µg/L	10.9 µg/L	109%
			3	1	12.1 µg/L	12.1 µg/L	121%
	Conc. 5	32.0 µg/L	1	1	38.8 µg/L	38.8 µg/L	121%
			3	1	36.4 µg/L	36.4 µg/L	114%
	Conc. 6	100 µg/L	1	4	28.8 µg/L	115 µg/L	115%
			3	4	26.4 µg/L	105 µg/L	105%
November 12, 2019	Control	0.00 µg/L	2	1	<LOQ*	<LOQ	-
			4	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	2	1	0.294 µg/L	0.294 µg/L	91.9%
			4	1	0.336 µg/L	0.336 µg/L	105%
	Conc. 2	1.00 µg/L	2	1	0.976 µg/L	0.976 µg/L	97.6%
			4	1	0.890 µg/L	0.890 µg/L	89.0%
	Conc. 3	3.20 µg/L	2	1	3.24 µg/L	3.24 µg/L	101%
			4	1	3.43 µg/L	3.43 µg/L	107%
	Conc. 4	10.0 µg/L	2	1	10.8 µg/L	10.8 µg/L	108%
			4	1	11.6 µg/L	11.6 µg/L	116%
	Conc. 5	32.0 µg/L	2	1	38.0 µg/L	38.0 µg/L	119%
			4	1	36.3 µg/L	36.3 µg/L	114%
	Conc. 6	100 µg/L	2	4	28.5 µg/L	114 µg/L	114%
			4	4	26.5 µg/L	106 µg/L	106%

Sampling, date and time (2019)	Treat-ment	Nominal Dexa-metha-sone conc.	Test vessel	Dilution factor, DF	LC-MS/MS quantific. data, CLC-MS/MS	Measured analyte conc., CW	Percent of nominal
November 18, 2019	Control	0.00 µg/L	1	1	<LOQ*	<LOQ	-
			3	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	1	1	0.294 µg/L	0.294 µg/L	91.9%
			3	1	0.363 µg/L	0.363 µg/L	113%
	Conc. 2	1.00 µg/L	1	1	0.872 µg/L	0.872 µg/L	87.2%
			3	1	0.881 µg/L	0.881 µg/L	88.1%
	Conc. 3	3.20 µg/L	1	1	3.17 µg/L	3.17 µg/L	99.0%
			3	1	3.23 µg/L	3.23 µg/L	101%
	Conc. 4	10.0 µg/L	1	1	10.7 µg/L	10.7 µg/L	107%
			3	1	12.5 µg/L	12.5 µg/L	125%
	Conc. 5	32.0 µg/L	1	1	36.2 µg/L	36.2 µg/L	113%
			3	1	35.4 µg/L	35.4 µg/L	111%
	Conc. 6	100 µg/L	1	4	26.2 µg/L	105 µg/L	105%
			3	4	25.7 µg/L	103 µg/L	103%
November 25, 2019	Control	0.00 µg/L	2	1	<LOQ*	<LOQ	-
			4	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	2	1	0.297 µg/L	0.297 µg/L	92.8%
			4	1	0.283 µg/L	0.283 µg/L	88.4%
	Conc. 2	1.00 µg/L	2	1	0.864 µg/L	0.864 µg/L	86.4%
			4	1	0.979 µg/L	0.979 µg/L	97.9%
	Conc. 3	3.20 µg/L	2	1	3.20 µg/L	3.20 µg/L	100%
			4	1	3.66 µg/L	3.66 µg/L	114%
	Conc. 4	10.0 µg/L	2	1	10.7 µg/L	10.7 µg/L	107%
			4	1	11.5 µg/L	11.5 µg/L	115%
	Conc. 5	32.0 µg/L	2	1	37.0 µg/L	37.0 µg/L	116%
			4	1	35.4 µg/L	35.4 µg/L	111%
	Conc. 6	100 µg/L	2	4	25.2 µg/L	101 µg/L	101%
			4	4	24.9 µg/L	99.7 µg/L	99.7%

Sampling, date and time (2019)	Treat-ment	Nominal Dexa-metha-sone conc.	Test vessel	Dilution factor, DF	LC-MS/MS quantific. data, CLC-MS/MS	Measured analyte conc., CW	Percent of nominal
December 02, 2019	Control	0.00 µg/L	1	1	<LOQ*	<LOQ	-
			3	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	1	1	0.292 µg/L	0.292 µg/L	91.3%
			3	1	0.272 µg/L	0.272 µg/L	85.0%
	Conc. 2	1.00 µg/L	1	1	0.955 µg/L	0.955 µg/L	95.5%
			3	1	0.921 µg/L	0.921 µg/L	92.1%
	Conc. 3	3.20 µg/L	1	1	3.21 µg/L	3.21 µg/L	100%
			3	1	3.39 µg/L	3.39 µg/L	106%
	Conc. 4	10.0 µg/L	1	1	n.d.**	<LOQ	-
			1	1	9.687 µg/L	9.69 µg/L	96.9%
			3	1	12.2 µg/L	12.2 µg/L	122%
	Conc. 5	32.0 µg/L	1	1	35.8 µg/L	35.8 µg/L	112%
			3	1	35.3 µg/L	35.3 µg/L	110%
	Conc. 6	100 µg/L	1	4	26.8 µg/L	107 µg/L	107%
			3	4	25.7 µg/L	103 µg/L	103%
December 09, 2019	Control	0.00 µg/L	2	1	<LOQ*	<LOQ	-
			4	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	2	1	0.278 µg/L	0.278 µg/L	86.9%
			4	1	0.264 µg/L	0.264 µg/L	82.5%
	Conc. 2	1.00 µg/L	2	1	0.893 µg/L	0.893 µg/L	89.3%
			4	1	0.838 µg/L	0.838 µg/L	83.8%
	Conc. 3	3.20 µg/L	2	1	2.88 µg/L	2.88 µg/L	89.9%
			4	1	2.97 µg/L	2.97 µg/L	92.9%
	Conc. 4	10.0 µg/L	2	1	10.3 µg/L	10.3 µg/L	103%
			4	1	11.1 µg/L	11.1 µg/L	111%
	Conc. 5	32.0 µg/L	2	1	38.8 µg/L	38.8 µg/L	121%
			4	1	34.0 µg/L	34.0 µg/L	106%
	Conc. 6	100 µg/L	2	4	27.4 µg/L	110 µg/L	110%
			4	4	25.8 µg/L	103 µg/L	103%

Sampling, date and time (2019)	Treat-ment	Nominal Dexa-metha-sone conc.	Test vessel	Dilution factor, DF	LC-MS/MS quantific. data, CLC-MS/MS	Measured analyte conc., CW	Percent of nominal
December 16, 2019	Control	0.00 µg/L	1	1	<LOQ*	<LOQ	-
			3	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	1	1	0.303 µg/L	0.303 µg/L	94.7%
			3	1	0.348 µg/L	0.348 µg/L	109%
	Conc. 2	1.00 µg/L	1	1	1.03 µg/L	1.03 µg/L	103%
			3	1	0.912 µg/L	0.912 µg/L	91.2%
	Conc. 3	3.20 µg/L	1	1	3.45 µg/L	3.45 µg/L	108%
			3	1	3.37 µg/L	3.37 µg/L	105%
	Conc. 4	10.0 µg/L	1	1	11.5 µg/L	11.5 µg/L	115%
			3	1	11.3 µg/L	11.3 µg/L	113%
	Conc. 5	32.0 µg/L	1	1	37.3 µg/L	37.3 µg/L	117%
			3	1	33.5 µg/L	33.5 µg/L	105%
	Conc. 6	100 µg/L	1	4	26.4 µg/L	106 µg/L	106%
			3	4	24.0 µg/L	95.9 µg/L	95.9%
December 23, 2019	Control	0.00 µg/L	2	1	<LOQ*	<LOQ	-
			4	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	2	1	0.336 µg/L	0.336 µg/L	105%
			4	1	0.386 µg/L	0.386 µg/L	121%
	Conc. 2	1.00 µg/L	2	1	0.989 µg/L	0.989 µg/L	98.9%
			4	1	1.02 µg/L	1.02 µg/L	102%
	Conc. 3	3.20 µg/L	2	1	3.47 µg/L	3.47 µg/L	108%
			4	1	3.57 µg/L	3.57 µg/L	111%
	Conc. 4	10.0 µg/L	2	1	10.4 µg/L	10.4 µg/L	104%
			4	1	11.7 µg/L	11.7 µg/L	117%
	Conc. 5	32.0 µg/L	2	1	37.6 µg/L	37.6 µg/L	118%
			4	1	33.1 µg/L	33.1 µg/L	104%
	Conc. 6	100 µg/L	2	4	27.4 µg/L	110 µg/L	110%
			4	4	24.7 µg/L	98.9 µg/L	98.9%

Sampling, date and time (2019)	Treat-ment	Nominal Dexa-metha-sone conc.	Test vessel	Dilution factor, DF	LC-MS/MS quantific. data, CLC-MS/MS	Measured analyte conc., CW	Percent of nominal
January 02, 2020	Control	0.00 µg/L	1	1	<LOQ*	<LOQ	-
			3	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	1	1	0.330 µg/L	0.330 µg/L	103%
			3	1	0.338 µg/L	0.338 µg/L	106%
	Conc. 2	1.00 µg/L	1	1	0.910 µg/L	0.910 µg/L	91.0%
			3	1	0.839 µg/L	0.839 µg/L	83.9%
	Conc. 3	3.20 µg/L	1	1	2.97 µg/L	2.97 µg/L	92.7%
			3	1	3.02 µg/L	3.02 µg/L	94.4%
					n.d.**	<LOQ	-
	Conc. 4	10.0 µg/L	1	1	10.5 µg/L	10.5 µg/L	105%
			3	1	11.5 µg/L	11.5 µg/L	115%
	Conc. 5	32.0 µg/L	1	1	37.8 µg/L	37.8 µg/L	118%
			3	1	33.0 µg/L	33.0 µg/L	103%
	Conc. 6	100 µg/L	1	4	25.4 µg/L	102 µg/L	102%
			3	4	24.1 µg/L	96.2 µg/L	96.2%
January 06, 2020	Control	0.00 µg/L	2	1	<LOQ*	<LOQ	-
			4	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	2	1	0.325 µg/L	0.325 µg/L	102%
			4	1	0.273 µg/L	0.273 µg/L	85.3%
	Conc. 2	1.00 µg/L	2	1	0.826 µg/L	0.826 µg/L	82.6%
			4	1	0.812 µg/L	0.812 µg/L	81.2%
	Conc. 3	3.20 µg/L	2	1	3.00 µg/L	3.00 µg/L	93.9%
			4	1	2.83 µg/L	2.83 µg/L	88.4%
	Conc. 4	10.0 µg/L	2	1	10.0 µg/L	10.0 µg/L	100%
			4	1	11.6 µg/L	11.6 µg/L	116%
	Conc. 5	32.0 µg/L	2	1	37.1 µg/L	37.1 µg/L	116%
			4	1	35.3 µg/L	35.3 µg/L	110%
	Conc. 6	100 µg/L	2	4	23.8 µg/L	95.3 µg/L	95.3%
			4	4	23.9 µg/L	95.7 µg/L	95.7%

Sampling, date and time (2019)	Treat-ment	Nominal Dexa-metha-sone conc.	Test vessel	Dilution factor, DF	LC-MS/MS quantific. data, CLC-MS/MS	Measured analyte conc., CW	Percent of nominal
January 13, 2020	Control	0.00 µg/L	1	1	<LOQ*	<LOQ	-
			3	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	1	1	0.258 µg/L	0.258 µg/L	80.6%
			3	1	0.332 µg/L	0.332 µg/L	104%
	Conc. 2	1.00 µg/L	1	1	0.969 µg/L	0.969 µg/L	96.9%
			3	1	0.999 µg/L	0.999 µg/L	99.9%
	Conc. 3	3.20 µg/L	1	1	3.26 µg/L	3.26 µg/L	102%
			3	1	3.22 µg/L	3.22 µg/L	101%
	Conc. 4	10.0 µg/L	1	1	10.8 µg/L	10.8 µg/L	108%
			3	1	11.3 µg/L	11.3 µg/L	113%
	Conc. 5	32.0 µg/L	1	1	33.4 µg/L	33.4 µg/L	104%
			3	1	32.0 µg/L	32.0 µg/L	100%
	Conc. 6	100 µg/L	1	4	25.3 µg/L	101 µg/L	101%
			3	4	23.6 µg/L	94.5 µg/L	94.5%
January 20, 2020	Control	0.00 µg/L	2	1	<LOQ*	<LOQ	-
			4	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	2	1	0.411 µg/L	0.411 µg/L	128%
			4	1	0.356 µg/L	0.356 µg/L	111%
	Conc. 2	1.00 µg/L	2	1	0.934 µg/L	0.934 µg/L	93.4%
			4	1	1.11 µg/L	1.11 µg/L	111%
	Conc. 3	3.20 µg/L	2	1	3.58 µg/L	3.58 µg/L	112%
			4	1	3.70 µg/L	3.70 µg/L	116%
	Conc. 4	10.0 µg/L	2	1	9.62 µg/L	9.62 µg/L	96.2%
			4	1	9.75 µg/L	9.75 µg/L	97.5%
	Conc. 5	32.0 µg/L	2	1	36.0 µg/L	36.0 µg/L	112%
			4	1	35.0 µg/L	35.0 µg/L	110%
	Conc. 6	100 µg/L	2	4	27.1 µg/L	109 µg/L	109%
			4	4	24.8 µg/L	99.3 µg/L	99.3%

Sampling, date and time (2019)	Treat-ment	Nominal Dexa-metha-sone conc.	Test vessel	Dilution factor, DF	LC-MS/MS quantific. data, CLC-MS/MS	Measured analyte conc., CW	Percent of nominal
January 27, 2020	Control	0.00 µg/L	1	1	<LOQ*	<LOQ	-
			3	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	1	1	0.331 µg/L	0.331 µg/L	103%
			3	1	0.373 µg/L	0.373 µg/L	117%
	Conc. 2	1.00 µg/L	1	1	0.938 µg/L	0.938 µg/L	93.8%
			3	1	1.04 µg/L	1.04 µg/L	104%
	Conc. 3	3.20 µg/L	1	1	3.52 µg/L	3.52 µg/L	110%
			3	1	3.24 µg/L	3.24 µg/L	101%
	Conc. 4	10.0 µg/L	1	1	9.44 µg/L	9.44 µg/L	94%
			3	1	9.60 µg/L	9.60 µg/L	96%
	Conc. 5	32.0 µg/L	1	1	33.5 µg/L	33.5 µg/L	105%
			3	1	31.0 µg/L	31.0 µg/L	97%
	Conc. 6	100 µg/L	1	4	24.8 µg/L	99.3 µg/L	99.3%
			3	4	22.6 µg/L	90.3 µg/L	90.3%
February 04, 2020	Control	0.00 µg/L	2	1	<LOQ*	<LOQ	-
			4	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	2	1	0.403 µg/L	0.403 µg/L	126%
			4	1	0.414 µg/L	0.414 µg/L	129%
	Conc. 2	1.00 µg/L	2	1	1.02 µg/L	1.015 µg/L	102%
			4	1	1.02 µg/L	1.024 µg/L	102%
	Conc. 3	3.20 µg/L	2	1	3.39 µg/L	3.39 µg/L	106%
			4	1	3.32 µg/L	3.32 µg/L	104%
	Conc. 4	10.0 µg/L	2	1	10.1 µg/L	10.1 µg/L	101%
			4	1	10.0 µg/L	10.0 µg/L	100%
	Conc. 5	32.0 µg/L	2	1	37.0 µg/L	37.0 µg/L	116%
			4	1	33.9 µg/L	33.9 µg/L	106%
	Conc. 6	100 µg/L	2	4	27.1 µg/L	108.4 µg/L	108%
			4	4	23.1 µg/L	92.5 µg/L	92.5%

Sampling, date and time (2019)	Treat-ment	Nominal Dexa-metha-sone conc.	Test vessel	Dilution factor, DF	LC-MS/MS quantific. data, CLC-MS/MS	Measured analyte conc., CW	Percent of nominal
February 10, 2020	Control	0.00 µg/L	1	1	<LOQ*	<LOQ	-
			3	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	1	1	0.348 µg/L	0.348 µg/L	109%
			3	1	0.321 µg/L	0.321 µg/L	100%
	Conc. 2	1.00 µg/L	1	1	0.720 µg/L	0.720 µg/L	72.0%
			3	1	1.04 µg/L	1.04 µg/L	104%
	Conc. 3	3.20 µg/L	1	1	3.41 µg/L	3.41 µg/L	107%
			3	1	3.29 µg/L	3.29 µg/L	103%
	Conc. 4	10.0 µg/L	1	1	10.4 µg/L	10.4 µg/L	104%
			3	1	9.97 µg/L	9.97 µg/L	99.7%
	Conc. 5	32.0 µg/L	1	1	37.1 µg/L	37.1 µg/L	116%
			3	1	34.2 µg/L	34.2 µg/L	107%
	Conc. 6	100 µg/L	1	4	25.7 µg/L	103 µg/L	103%
			3	4	24.9 µg/L	99.6 µg/L	99.6%
February 17, 2020	Control	0.00 µg/L	2	1	<LOQ*	<LOQ	-
			4	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	2	1	0.348 µg/L	0.348 µg/L	109%
			4	1	0.404 µg/L	0.404 µg/L	126%
	Conc. 2	1.00 µg/L	2	1	0.603 µg/L	0.603 µg/L	60.3%
			4	1	1.04 µg/L	1.04 µg/L	104%
	Conc. 3	3.20 µg/L	2	1	3.30 µg/L	3.30 µg/L	103%
			4	1	3.22 µg/L	3.22 µg/L	100%
	Conc. 4	10.0 µg/L	2	1	9.98 µg/L	10.0 µg/L	99.8%
			4	1	10.2 µg/L	10.2 µg/L	102%
	Conc. 5	32.0 µg/L	2	1	34.7 µg/L	34.7 µg/L	108%
			4	1	30.9 µg/L	30.9 µg/L	96.5%
	Conc. 6	100 µg/L	2	4	27.1 µg/L	108 µg/L	108%
			4	4	25.1 µg/L	100 µg/L	100%

Sampling, date and time (2019)	Treat-ment	Nominal Dexa-metha-sone conc.	Test vessel	Dilution factor, DF	LC-MS/MS quantific. data, CLC-MS/MS	Measured analyte conc., CW	Percent of nominal
February 24, 2020	Control	0.00 µg/L	1	1	<LOQ*	<LOQ	-
			3	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	1	1	0.407 µg/L	0.407 µg/L	127%
			3	1	0.345 µg/L	0.345 µg/L	108%
	Conc. 2	1.00 µg/L	1	1	0.546 µg/L	0.546 µg/L	54.6%
			3	1	0.965 µg/L	0.965 µg/L	96.5%
	Conc. 3	3.20 µg/L	1	1	3.35 µg/L	3.35 µg/L	105%
			3	1	3.08 µg/L	3.08 µg/L	96.2%
	Conc. 4	10.0 µg/L	1	1	9.92 µg/L	9.92 µg/L	99.2%
			3	1	10.8 µg/L	10.8 µg/L	108%
	Conc. 5	32.0 µg/L	1	1	35.8 µg/L	35.8 µg/L	112%
			3	1	33.9 µg/L	33.9 µg/L	106%
	Conc. 6	100 µg/L	1	4	26.4 µg/L	106 µg/L	106%
			3	4	24.6 µg/L	98.4 µg/L	98.4%
March 02, 2020	Control	0.00 µg/L	2	1	<LOQ*	<LOQ	-
			4	1	<LOQ*	<LOQ	-
	Conc.1	0.32 µg/L	2	1	0.351 µg/L	0.351 µg/L	110%
			4	1	0.308 µg/L	0.308 µg/L	96.3%
	Conc. 2	1.00 µg/L	2	1	1.49 µg/L	1.49 µg/L	149%
			4	1	1.14 µg/L	1.14 µg/L	114%
	Conc. 3	3.20 µg/L	2	1	3.21 µg/L	3.21 µg/L	100%
			4	1	3.13 µg/L	3.13 µg/L	97.9%
	Conc. 4	10.0 µg/L	2	1	10.3 µg/L	10.3 µg/L	103%
			4	1	10.6 µg/L	10.6 µg/L	106%
	Conc. 5	32.0 µg/L	2	1	37.3 µg/L	37.3 µg/L	117%
			4	1	35.7 µg/L	35.7 µg/L	112%
	Conc. 6	100 µg/L	2	4	30.6 µg/L	122 µg/L	122%
			4	4	25.8 µg/L	103 µg/L	103%

*The LOQ was set at 0.10 µg/L for the measured concentration.
** Samples showed no recovery of test item. To insure correct exposure was re-established samples were taken and
analysed the day after. The result is shown directly after the initial value.

Daphnia magna reproduction test: All 42 samples were analysed. Results are presented in the table below.

Sampling Date	Test vessel	Nominal Conc.	Dilution Factor	LC-MS/MS quant. Data cLC-MS/MS	Measured analyte conc. cw	Percent of nominal
January 22, 2020 day 0 fresh	Control	0.00 µg/L	1	<LOQ*	<LOQ	-
	Solvent Control	0.00 µg/L	1	<LOQ*	<LOQ	-
	Conc.1	1.00 µg/L	1	1.08 µg/L	1.08 µg/L	108%
	Conc.2	3.20 µg/L	1	3.54 µg/L	3.54 µg/L	111%
	Conc.3	10.0 µg/L	1	11.2 µg/L	11.2 µg/L	112%
	Conc. 4	32.0 µg/L	1	35.6 µg/L	35.6 µg/L	111%
	Conc. 5	100 µg/L	4	28.4 µg/L	113 µg/L	113%
January 24, 2020 day 2 expo.	Control	0.00 µg/L	1	<LOQ*	<LOQ	-
	Solvent Control	0.00 µg/L	1	<LOQ*	<LOQ	-
	Conc.1	1.00 µg/L	1	1.15 µg/L	1.15 µg/L	115%
	Conc.2	3.20 µg/L	1	3.40 µg/L	3.40 µg/L	106%
	Conc.3	10.0 µg/L	1	10.3 µg/L	10.3 µg/L	103%
	Conc. 4	32.0 µg/L	1	34.6 µg/L	34.6 µg/L	108%
	Conc. 5	100 µg/L	4	26.9 µg/L	107 µg/L	107%
January 31, 2020 day 9 fresh	Control	0.00 µg/L	1	<LOQ*	<LOQ	-
	Solvent Control	0.00 µg/L	1	<LOQ*	<LOQ	-
	Conc.1	1.00 µg/L	1	1.11 µg/L	1.11 µg/L	111%
	Conc.2	3.20 µg/L	1	3.58 µg/L	3.58 µg/L	112%
	Conc.3	10.0 µg/L	1	10.7 µg/L	10.7 µg/L	107%
	Conc. 4	32.0 µg/L	1	34.8 µg/L	34.8 µg/L	109%
	Conc. 5	100 µg/L	4	27.1 µg/L	108 µg/L	108%
February 03, 2020 day 12 expo.	Control	0.00 µg/L	1	<LOQ*	<LOQ	-
	Solvent Control	0.00 µg/L	1	<LOQ*	<LOQ	-

Sampling Date	Test vessel	Nominal Conc.	Dilution Factor	LC-MS/MS quant. Data cLC-MS/MS	Measured analyte conc. cw	Percent of nominal
	Conc.1	1.00 µg/L	1	1.10 µg/L	1.10 µg/L	110%
	Conc.2	3.20 µg/L	1	3.41 µg/L	3.41 µg/L	107%
	Conc.3	10.0 µg/L	1	10.3 µg/L	10.3 µg/L	103%
	Conc. 4	32.0 µg/L	1	33.9 µg/L	33.9 µg/L	106%
	Conc. 5	100 µg/L	4	27.1 µg/L	108 µg/L	108%
February 10, 2020 day 19 fresh	Control	0.00 µg/L	1	<LOQ*	<LOQ	-
	Solvent Control	0.00 µg/L	1	<LOQ*	<LOQ	-
	Conc.1	1.00 µg/L	1	1.00 µg/L	0.996 µg/L	99.6%
	Conc.2	3.20 µg/L	1	3.26 µg/L	3.26 µg/L	102%
	Conc.3	10.0 µg/L	1	10.7 µg/L	10.7 µg/L	107%
	Conc. 4	32.0 µg/L	1	34.4 µg/L	34.4 µg/L	107%
	Conc. 5	100 µg/L	4	26.5 µg/L	106 µg/L	106%
February 12, 2020 day 21 expo.	Control	0.00 µg/L	1	<LOQ*	<LOQ	-
	Solvent Control	0.00 µg/L	1	<LOQ*	<LOQ	-
	Conc.1	1.00 µg/L	1	0.986 µg/L	0.986 µg/L	98.6%
	Conc.2	3.20 µg/L	1	3.33 µg/L	3.33 µg/L	104%
	Conc.3	10.0 µg/L	1	10.7 µg/L	10.7 µg/L	107%
	Conc. 4	32.0 µg/L	1	34.5 µg/L	34.5 µg/L	108%
	Conc. 5	100 µg/L	4	26.1 µg/L	105 µg/L	105%

* The LOQ was set at 0.1 µg/L as the measured concentration.

Quality control

The QC samples were processed and measured twenty-seven times during the course of the study. For the QC samples at level 1, with a nominal concentration of 2.00 µg/L, the mean recovery was 100% with an RSD value of 3.18%, and for level 2, with a nominal concentration of 40.0 µg/L, the mean recovery was 102% with an RSD value of 2.69%.

In the Daphnia study the recovery values for QC samples (5 at each level) were as follows. For samples at level 1, with a nominal concentration of 2.00 µg/L, the mean recovery was 99.2% with an RSD value of 4.42%, and for level 2, with a nominal concentration of 40.0 µg/L, the mean recovery was 103% with an RSD value of 1.28%.

The quality control data show that the measurements were done with high accuracy and precision over the entire run time of the study.

B.2.7 Representative LC-MS/MS chromatograms

Typical LC-MS/MS chromatograms of calibration samples, controls and test media samples are shown in Figure 22 to Figure 29.

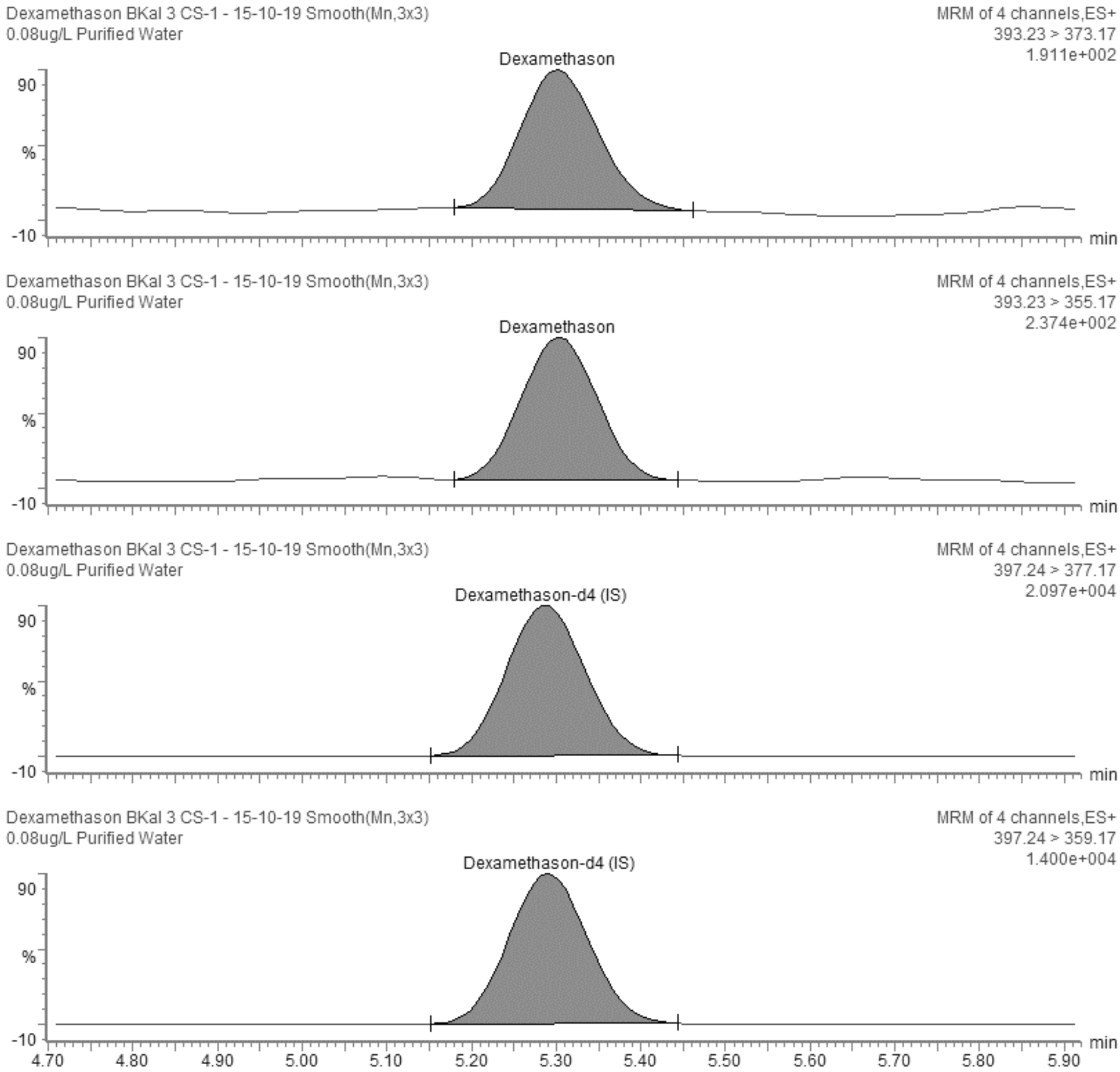
Each figure shows four ion chromatograms in stacked windows (top-down):

Ion chromatogram	Mass transition
Dexamethasone, quantification ion	m/z 393.23 → m/z 373.17
Dexamethasone, qualifier ion	m/z 393.23 → m/z 355.17
Dexamethasone-d4 (IS), quantification ion	m/z 397.24 → m/z 377.17
Dexamethasone-d4 (IS), qualifier ion	m/z 397.24 → m/z 359.17

The dashed line in some chromatograms shows the baseline of the integrated peaks executed by automatic integration using the Waters QuanLynx software. However, the grey highlighted part of the chromatographic peak reflects the manually integrated peak area; this corrected peak area was used for quantification of the analyte.

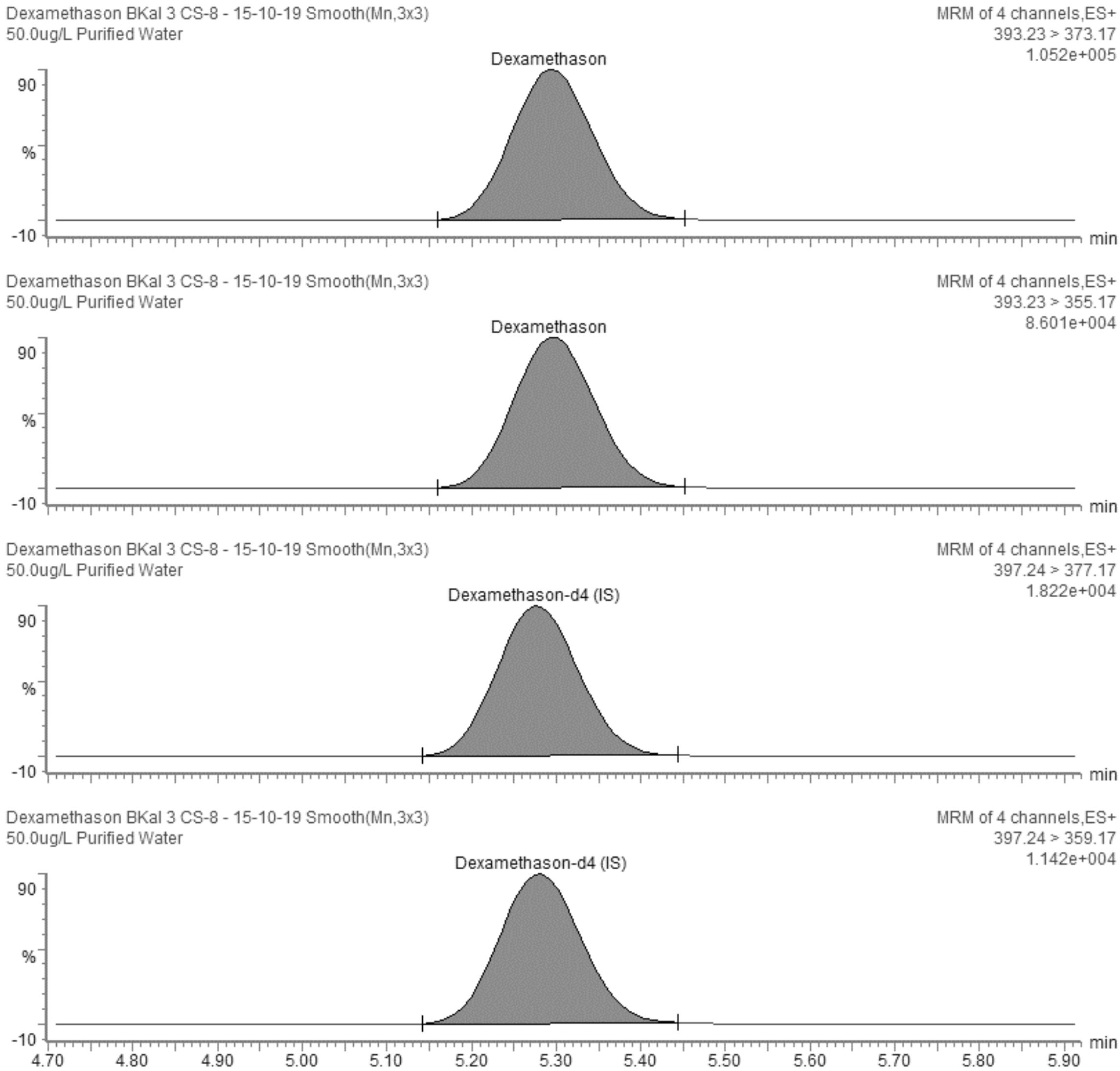
The retention time (t_R) for Dexamethasone was approximately 5.3 min.

Figure 22: Calibration sample CS-1 measured Oct. 15, 2019; Dexamethasone conc.: 0.08 µg/L.



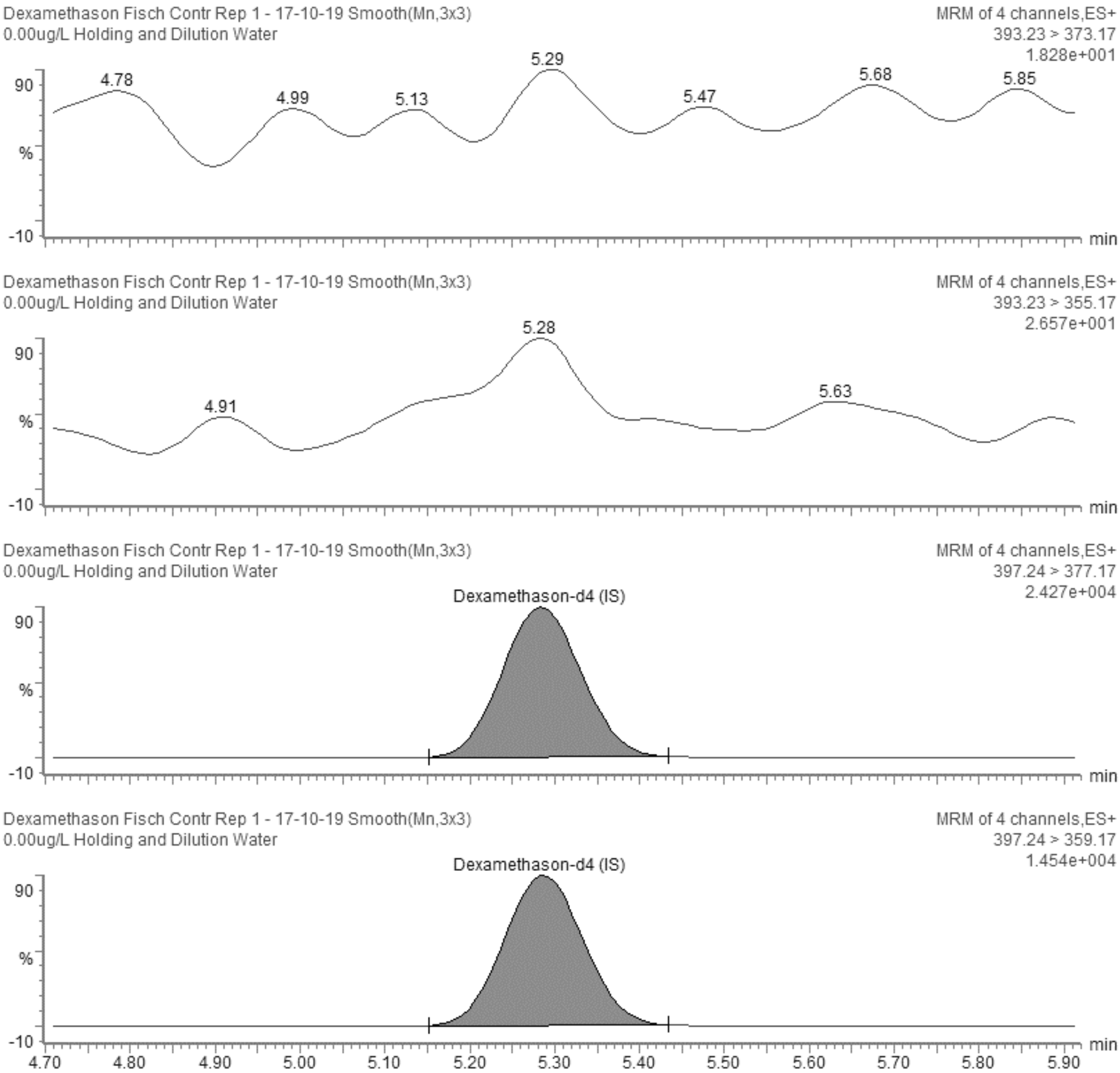
Source: Own graphic, Fraunhofer IME

Figure 23: Calibration sample CS-8 measured Oct. 15, 2019; Dexamethasone conc.: 50.0 µg/L.



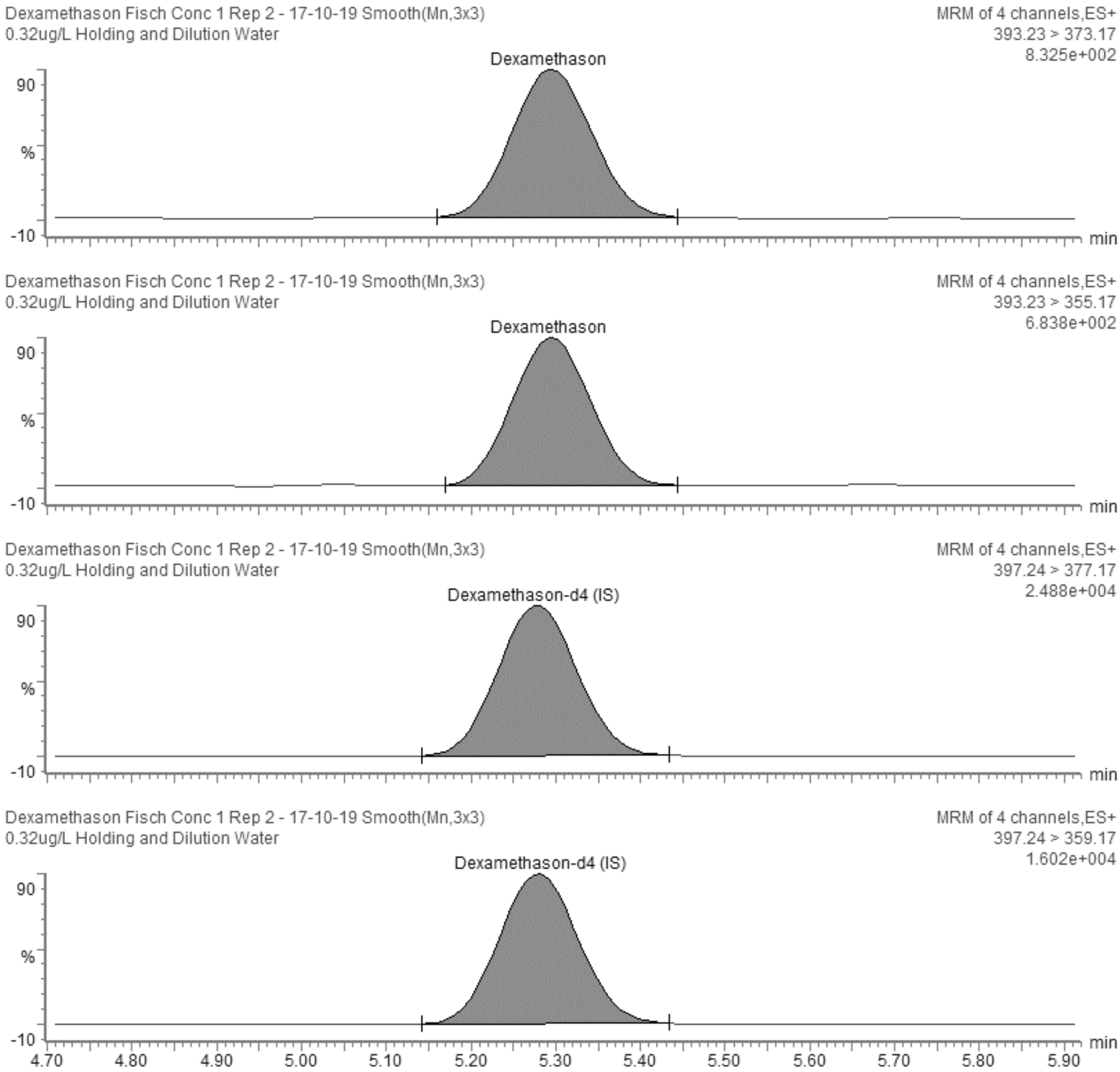
Source: Own graphic, Fraunhofer IME

Figure 24: Control sample, sampling time: test start (Oct. 17, 2019).



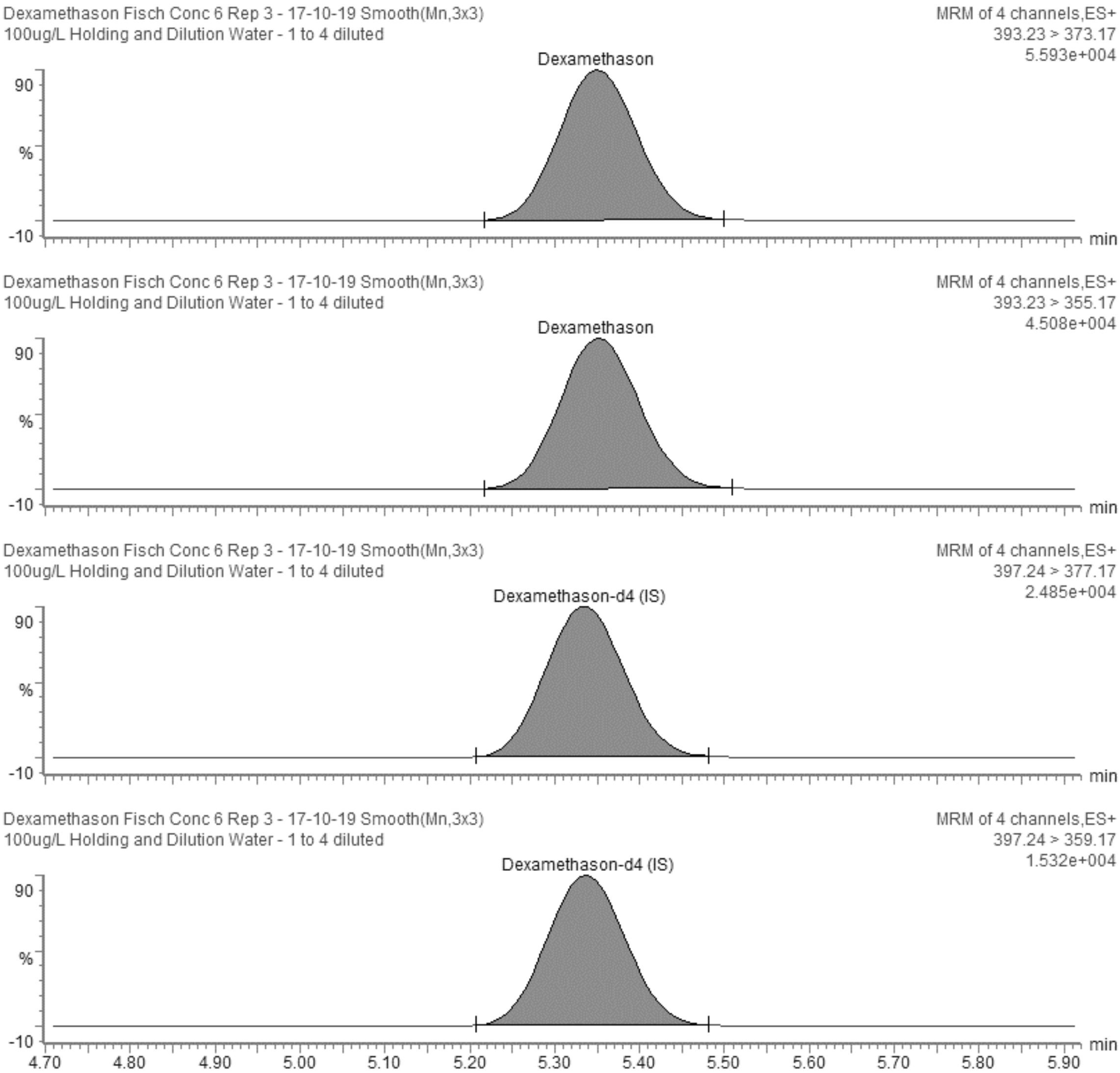
Source: Own graphic, Fraunhofer IME

Figure 25: Test media sample, nominal conc.: 0.32 µg/L, test start (Oct. 17, 2019).



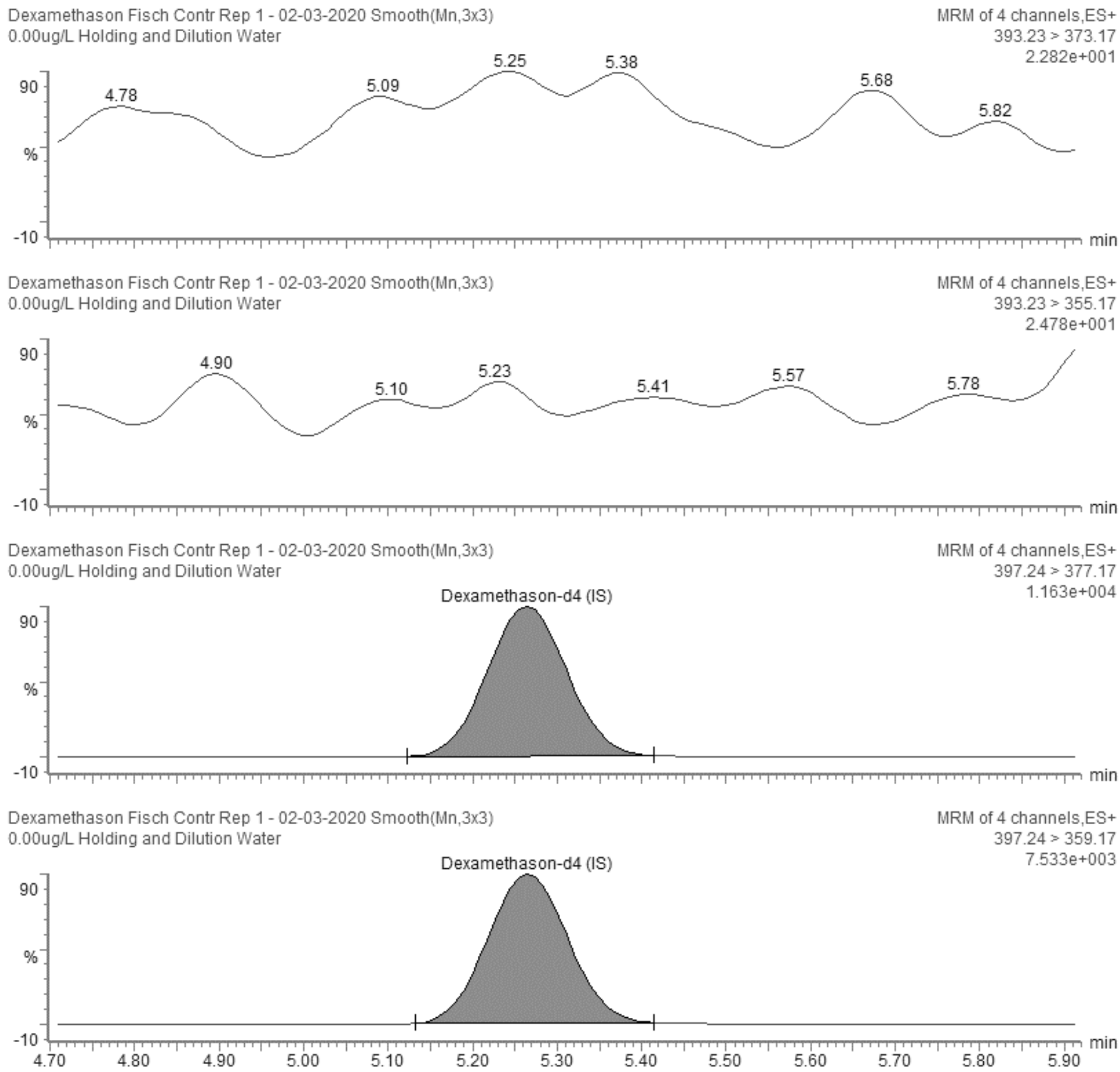
Source: Own graphic, Fraunhofer IME

Figure 26: Test media sample, nominal conc.: 100 µg/L, test start (Oct. 17, 2019).



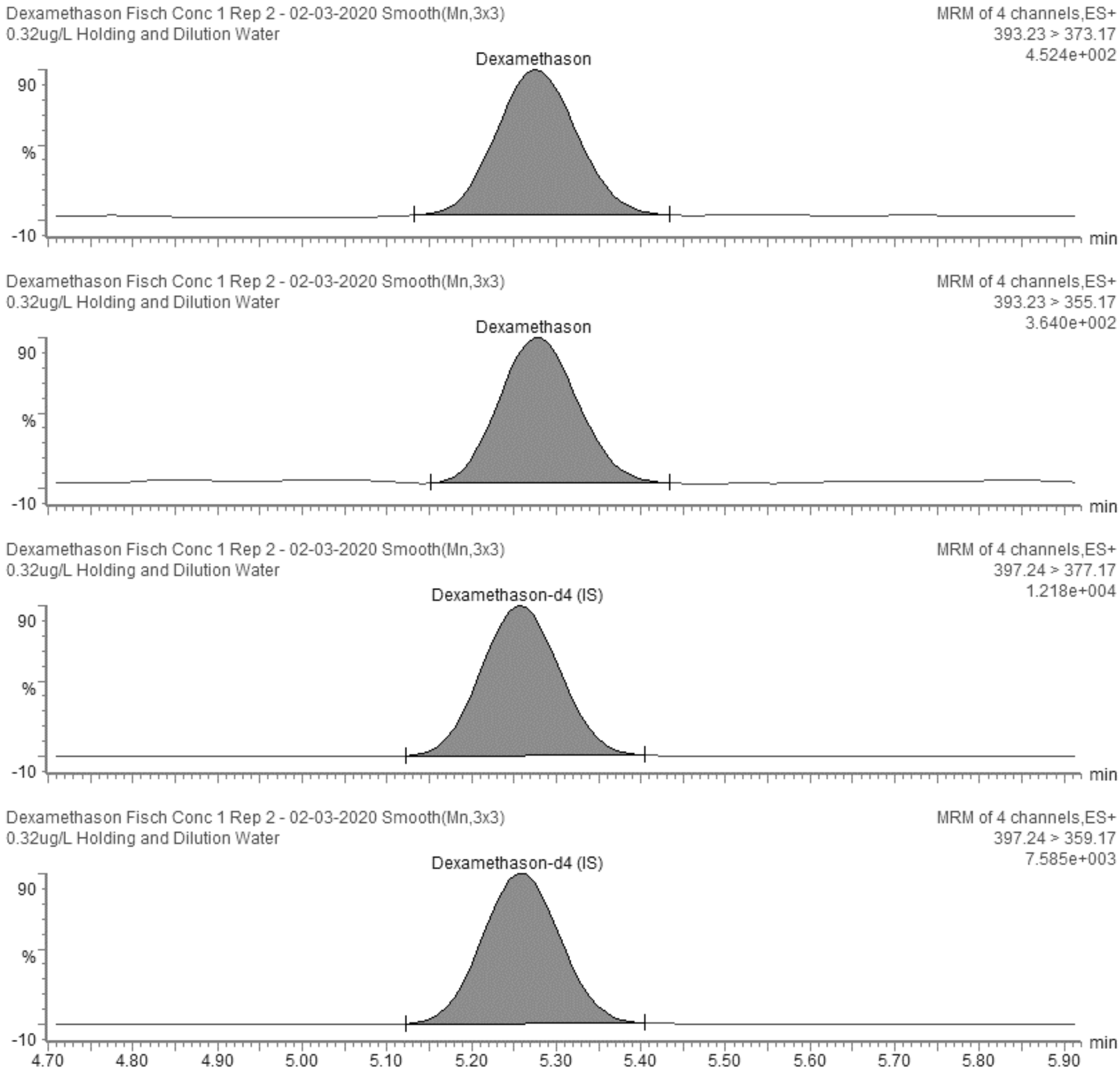
Source: Own graphic, Fraunhofer IME

Figure 27: Control sample, sampling time: test end (March 02, 2020).



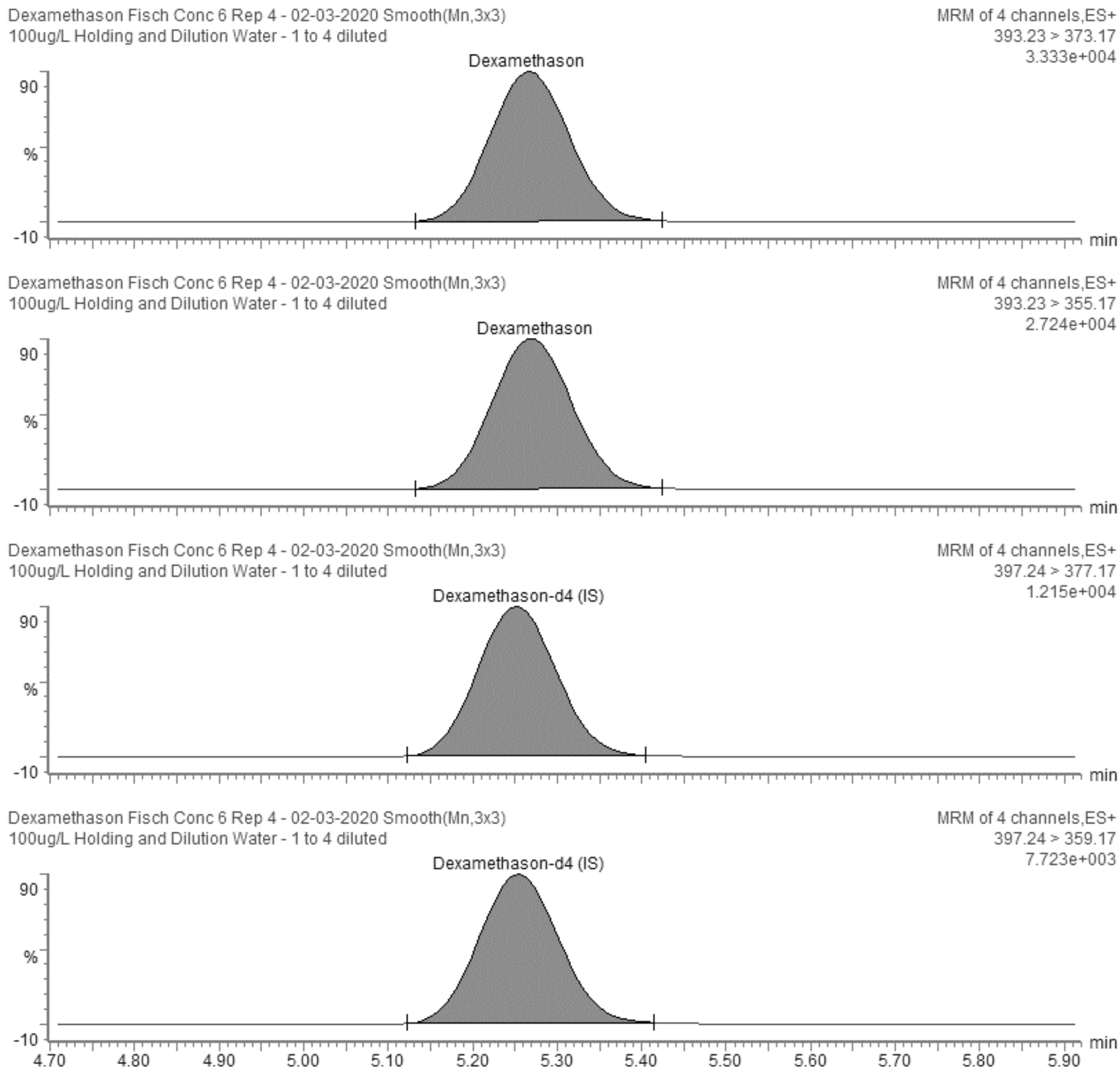
Source: Own graphic, Fraunhofer IME

Figure 28: Test medium sample, nominal conc.: 0.32 µg/L, test end (March 02, 2020).



Source: Own graphic, Fraunhofer IME

Figure 29: Test medium sample, nominal conc.: 100 µg/L, test end (March 02, 2020).



Source: Own graphic, Fraunhofer IME

B.2.8 Certificate of Analysis Dexamethasone

SIGMA-ALDRICH

3050 Spruce Street, Saint Louis, MO 63103 USA
Email USA: techserv@sial.com Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:	DEXAMETHASONE >= 98 % HPLC, powder
Product Number:	D1756
Batch Number:	BCBW7684
Brand:	Sigma
CAS Number:	50-02-2
Formula:	$C_{22}H_{29}FO_5$
Formula Weight:	392.46
Storage Temperature:	2-8 C
Quality Release Date:	23 MAR 2018
Recommended Retest Date:	FEB 2023

TEST	SPECIFICATION	RESULT
APPEARANCE (COLOR)	WHITE TO OFF WHITE	WHITE
APPEARANCE (FORM)	POWDER	POWDER
PURITY (HPLC AREA %)	≥ 98 %	100 %
SOLUBILITY (COLOR)	COLORLESS	COLORLESS
SOLUBILITY (TURBIDITY)	CLEAR	CLEAR
SOLUBILITY (METHOD)	25MG/ML OF METHANOL	25MG/ML OF METHANOL
INFRARED SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS
WAVELENGTH (1) (UV)	LAMBDA MAX 239 TO 241 NM	LAMBDA MAX 239 NM
MOLAR ABSORBANCY INDEX (1)	EMM = 15.0 TO 15.5	EMM = 15.1
SOLVENT (UV)	METHANOL	METHANOL

Dr. Reinhold Schwenninger
Quality Assurance
Buchs, Switzerland

Sigma-Aldrich Certificate of Analysis - Product D1756 Lot BCBW7684 Page 1 of 1

B.2.9 Certificate of Analysis Dexamethasone-d4 (Internal Standard)

CDN ISOTOPES

CERTIFICATE OF ANALYSIS

PRODUCT NAME	Dexamethasone-4,6α,21,21-d_4
PRODUCT NO.	D-5559
BULK LOT NO.	X-473
CAS #	N/A
MOLECULAR FORMULA	$C_{22}H_{25}D_4FO_5$
MOLECULAR WEIGHT	396.49

N.M.R.
OK (also 6.5%-d1 deuterated on position C-2)

HPLC
98.2% Chemical purity

Mass Analysis
97.9%-d4

Melting Point
246-248°C

T.L.C.
Single spot

Ying Cao, M.Sc.
Quality Control Manager

Expiration: ***Stable if stored under recommended conditions (see section 7 of SDS). After three years, the compound should be re-analysed for chemical purity before use.***

C/D/N ISOTOPES INC. - 88 Leacock Street - Pointe-Claire - Quebec - Canada - H9R 1H1
Tel. (514) 697-6254 (800) 565-4696 Fax (514) 697-6148 www.cdnisotopes.com sales@cdnisotopes.com

C Appendix

C.1 Ecotoxicological Studies with Dienogest: Zebrafish extended one generation reproduction test (ZEOGRT)

C.1.1 Test conditions: water temperature

Table 18: Water temperature [°C]; control and 3.20 ng Dienogest/L

	Nominal concentration Dienogest [ng/L]							
	Control				3.20			
Replicate	A	B	C	D	A	B	C	D
Time of exposure [day]	Water temperature [°C]							
0	26.9	26.8	26.6	26.6	26.8	26.8	26.6	26.6
1	27.5	27.4	27.0	26.8(*)	27.5	27.5	26.6	26.6
2	27.4	27.4	27.3	27.4	27.3	27.4	27.1	27.2
3	27.5	27.6(*)	28.0(*)	28.0(*)	27.6(*)	27.5	27.9(*)	27.9(*)
6	27.0	27.0	27.1	27.2	27.0	27.0	27.2	27.1
7	27.4	27.4	27.6(*)	27.6(*)	27.3	27.3	27.6(*)	27.6(*)
8	27.4	27.4	27.7(*)	27.7(*)	27.4	27.5	27.7(*)	27.8(*)
9	27.1	27.1	27.4	27.4	27.1	27.2	27.4	27.4
10	27.2	27.2	27.6(*)	27.6(*)	27.2	27.2	27.6(*)	27.7(*)
13	26.8	26.8	26.7	26.7	26.7	26.7	26.9	26.8
14	27.0	27.0	27.0	27.0	26.8	26.9	27.0	27.0
15	27.0	27.0	27.0	27.0	26.8	26.9	27.0	27.0
16	26.8	26.8	26.8	26.8	26.7	26.8	26.9	26.9
17	26.8	26.8	26.8	26.8	26.8	26.8	26.8	26.8
20	26.8	26.8	26.8	26.8	26.6	26.7	26.8	26.8
21	26.7	26.7	26.9	26.9	26.6	26.6	26.8	26.8
22	26.8	26.8	26.9	26.8	26.6	26.7	26.8	26.8
23	26.8	26.9	27.1	27.1	26.7	26.7	27.0	27.0
24	26.9	27.0	27.2	27.2	26.9	26.9	27.2	27.2
27	26.7	26.8	27.1	27.1	26.7	26.7	27.1	27.1
28	26.7	26.7	27.0	27.0	26.7	26.7	27.0	27.0
29	26.8	26.9	27.2	27.2	26.7	26.7	27.1	27.1

	Nominal concentration Dienogest [ng/L]							
30	26.8	26.9	27.2	27.2	26.7	26.7	27.1	27.1
31	26.8	26.8	27.1	27.1	26.8	26.7	27.1	27.1
34	26.5	26.7	26.9	27.0	26.6	26.5	26.6	26.7
35	26.5	26.6	26.9	27.0	26.6	26.6	26.7	26.7
36	26.6	26.7	27.0	27.0	26.6	26.7	26.7	26.7
37	26.6	26.6	27.0	26.9	26.6	26.6	26.7	26.7
38	26.6	26.8	26.8	27.0	26.8	26.8	26.6	26.7
41	26.6	26.7	26.8	26.9	26.6	26.6	26.3	26.4
42	26.6	26.7	26.8	27.0	26.6	26.7	26.4	26.5
43	26.7	26.8	26.8	26.9	26.6	26.7	26.4	26.4
44	26.6	26.7	26.8	26.9	26.6	26.7	26.4	26.5
45	26.7	26.7	26.8	26.9	26.6	26.7	26.4	26.4
48	26.6	26.7	26.8	26.9	26.7	26.6	26.4	26.6
49	26.6	26.7	26.8	27.0	26.6	26.6	26.5	26.6
50	26.6	26.6	26.9	27.0	26.6	26.5	26.5	26.6
51	26.5	26.5	26.7	26.8	26.5	26.4	26.5	26.6
52	26.5	26.5	26.7	26.7	26.5	26.4	26.4	26.5
55	26.8	26.8	26.4	26.6	26.7	26.7	26.3	26.3
56	26.7	26.7	26.4	26.5	26.5	26.5	26.3	26.3
57	26.7	26.8	26.6	26.7	26.7	26.7	26.4	26.3
58	26.6	26.8	26.7	26.8	26.7	26.8	26.4	26.4
59	26.6	26.6	26.5	26.6	26.5	26.5	26.3	26.3
62	26.7	26.7	26.3	26.3	26.6	26.5	26.6	26.6
63	26.7	26.7	26.4	26.4	26.6	26.5	26.6	26.6
64	26.7	26.6	26.4	26.4	26.6	26.5	26.7	26.5
65	26.7	26.7	26.5	26.5	26.6	26.5	26.7	26.5
66	26.8	26.8	26.8	26.7	26.8	26.7	26.9	26.8
69	26.8	26.8	26.7	26.6	26.7	26.6	26.7	26.8
70	26.8	26.8	26.7	26.6	26.6	26.6	26.7	26.7
71	26.7	26.7	26.6	26.5	26.6	26.5	26.6	26.6
72	27.0	27.0	26.7	26.5	27.0	26.9	27.0	26.9
73	27.1	27.1	26.8	26.6	27.0	27.0	27.2	27.1

	Nominal concentration Dienogest [ng/L]							
76	26.9	26.9	26.5	26.4	26.9	26.8	26.9	26.8
77	26.9	27.0	26.8	26.7	26.9	26.8	26.9	26.9
78	27.0	27.1	26.8	26.7	26.9	26.8	26.8	26.8
79	26.9	26.9	26.7	26.6	26.9	26.8	26.9	26.8
80	27.0	27.1	26.8	26.6	27.0	26.9	27.0	27.0
83	26.8	26.8	26.6	26.6	26.8	26.7	26.8	26.8
84	26.8	26.8	26.6	26.5	26.8	26.7	26.9	26.9
85	26.8	26.8	26.6	26.6	26.8	26.8	26.9	26.9
86	26.8	26.8	26.5	26.5	26.8	26.7	26.8	26.9
87	26.8	26.8	26.5	26.5	26.8	26.7	26.8	26.9
90	26.8	26.7	26.5	26.4	26.7	26.6	26.9	26.7
91	26.6	26.6	26.3	26.3	26.7	26.5	26.7	26.6
92	26.6	26.6	26.3	26.3	26.6	26.5	26.6	26.5
93	26.6	26.6	26.4	26.4	26.6	26.5	26.7	26.7
94	26.4	26.5	26.2	26.1	26.4	26.4	26.6	26.5
97	26.5	26.5	26.5	26.5	26.5	26.4	26.8	26.8
98	26.5	26.5	26.4	26.3	26.4	26.4	26.7	26.6
99	26.4	26.6	26.3	26.4	26.7	26.7	26.7	26.7
100	26.4	26.4	26.1	26.1	26.4	26.4	26.4	26.3
101	26.7	26.8	26.2	26.3	26.7	26.7	26.5	26.4
104	26.6	26.7	26.2	26.3	26.7	26.6	26.6	26.4
105	26.6	26.7	26.3	26.4	26.7	26.7	26.6	26.5
106	26.6	26.7	26.3	26.3	26.6	26.6	26.6	26.5
107	26.6	26.6	26.2	26.2	26.7	26.6	26.5	26.4
108	26.7	26.7	26.4	26.4	26.9	26.8	26.6	26.5
111	26.7	26.7	26.4	26.4	26.8	26.7	26.5	26.4
112	26.8	26.8	26.5	26.5	26.9	26.8	26.6	26.5
113	26.7	26.7	26.4	26.4	26.8	26.7	26.6	26.5
114	26.7	26.7	26.4	26.4	26.8	26.8	26.6	26.5
115	26.7	26.7	26.3	26.3	26.8	26.7	26.6	26.5
118	26.9	26.8	26.4	26.4	26.9	26.8	26.7	26.5
119	26.9	26.8	26.4	26.4	26.7	26.7	26.5	26.4

	Nominal concentration Dienogest [ng/L]							
120	26.8	26.7	26.3	26.3	26.7	26.7	26.5	26.4
121	26.6	26.5	26.4	26.4	26.5	26.6	26.6	26.6
122	26.7	26.7	26.7	26.8	26.7	26.7	26.7	26.7
125	26.4	26.4	26.7	26.7	26.4	26.5	26.5	26.6
126	26.4	26.4	26.7	26.8	26.4	26.5	26.4	26.5
127	26.5	26.6	26.6	26.7	26.5	26.5	26.4	26.5
128	-	-	-	-	-	-	26.5	26.6
129	-	-	-	-	-	-	-	-
Mean	**26.8**	**26.8**	**26.7**	**26.7**	**26.7**	**26.7**	**26.7**	**26.7**
SD	0.2	0.2	0.4	0.4	0.2	0.2	0.3	0.3
RSD	0.9	0.8	1.3	1.4	0.9	0.9	1.2	1.2
Min	26.4	26.4	26.1	26.1	26.4	26.4	26.3	26.3
Max	27.5	27.6	28.0	28.0	27.6	27.5	27.9	27.9

(*) During the initial phase of the study with Dienogest. The water temperature was exceeded on single days. After technical adjustment. The temperature was in the recommended range until the end of the study.

Table 19: Water temperature [°C]; 10.0 and 32.0 ng Dienogest/L

	Nominal concentration Dienogest [ng/L]							
	10.0				32.0			
Replicate	A	B	C	D	A	B	C	D
Time of exposure [day]	Water temperature [°C]							
0	26.6	26.6	26.6	26.6	26.8	26.9	26.8	26.8
1	27.4	27.4	26.7	26.7	26.8	26.9	26.8	26.9
2	27.3	27.4	27.3	27.3	27.5	27.5	27.4	27.3
3	27.4	27.5	28.0(*)	28.0(*)	27.5	27.7(*)	28.0(*)	28.0(*)
6	27.0	27.0	27.2	27.2	27.1	27.3	27.2	27.3
7	27.3	27.4	27.6(*)	27.7(*)	27.5	27.6(*)	27.7(*)	27.7(*)
8	27.4	27.6(*)	27.7(*)	27.8(*)	27.6(*)	27.7(*)	27.8(*)	27.7(*)
9	27.1	27.2	27.4	27.5	27.2	27.3	27.5	27.5
10	27.2	27.3	27.7(*)	27.7(*)	27.2	27.3	27.6(*)	27.7(*)
13	26.7	26.8	26.8	26.9	26.8	26.9	26.8	26.9
14	26.8	26.9	26.9	27.0	26.9	27.0	26.9	27.0
15	26.8	26.9	27.0	27.0	26.9	27.0	26.9	27.0
16	26.7	26.8	26.9	26.9	26.8	26.9	27.0	27.0
17	26.7	26.7	26.8	26.8	26.8	26.9	26.8	26.9
20	26.7	26.7	26.8	26.9	26.8	26.9	26.8	26.9
21	26.6	26.6	26.9	27.0	27.0	26.9	27.0	27.0
22	26.7	26.7	26.9	27.0	26.9	26.9	27.0	27.0
23	26.7	26.7	27.0	27.0	26.8	26.8	27.0	27.0
24	26.8	26.9	27.1	27.1	26.9	27.0	27.2	27.2
27	26.6	26.7	27.0	27.0	26.7	26.8	26.8	26.8
28	26.6	26.7	27.0	27.0	26.7	26.8	26.7	26.8
29	26.7	26.8	27.0	27.1	26.8	26.9	26.9	27.0
30	26.7	26.8	27.1	27.1	26.8	26.9	27.0	27.0
31	26.7	26.8	27.1	27.1	26.8	26.9	27.0	27.1
34	26.5	26.5	26.6	26.6	26.6	26.6	26.6	26.6
35	26.5	26.5	26.7	26.6	26.6	26.6	26.6	26.7
36	26.5	26.5	26.7	26.6	26.7	26.7	26.6	26.7
37	26.5	26.6	26.6	26.6	26.6	26.6	26.7	26.7

	Nominal concentration Dienogest [ng/L]							
38	26.7	26.8	26.6	26.6	26.9	27.0	26.7	26.7
41	26.7	26.7	26.4	26.4	26.8	26.9	26.6	26.6
42	26.6	26.8	26.4	26.5	26.8	26.9	26.6	26.6
43	26.6	26.7	26.4	26.5	26.8	26.9	26.6	26.6
44	26.6	26.7	26.4	26.5	26.8	26.9	26.6	26.6
45	26.6	26.7	26.4	26.5	26.7	26.9	26.6	26.6
48	26.6	26.7	26.4	26.5	26.8	27.0	26.4	26.4
49	26.5	26.6	26.4	26.5	26.7	26.9	26.4	26.4
50	26.6	26.5	26.4	26.4	26.7	26.9	26.5	26.5
51	26.5	26.4	26.4	26.4	26.7	26.8	26.4	26.5
52	26.5	26.4	26.4	26.4	26.7	26.8	26.5	26.5
55	26.6	26.7	26.4	26.3	26.7	26.8	26.4	26.4
56	26.6	26.7	26.4	26.3	26.5	26.6	26.3	26.3
57	26.7	26.8	26.4	26.4	26.7	26.8	26.4	26.4
58	26.7	26.8	26.4	26.4	26.8	26.9	26.4	26.4
59	26.5	26.6	26.3	26.3	26.8	26.9	26.5	26.5
62	26.4	26.5	26.6	26.6	26.7	27.0	26.7	26.7
63	26.5	26.6	26.5	26.6	26.7	27.0	26.7	26.7
64	26.5	26.5	26.6	26.6	26.6	26.9	26.7	26.7
65	26.5	26.5	26.6	26.6	26.6	26.9	26.7	26.7
66	26.7	26.7	26.9	27.0	26.9	27.2	27.1	27.1
69	26.6	26.6	26.7	26.8	26.7	27.0	26.7	26.8
70	26.6	26.6	26.7	26.8	26.8	27.0	26.7	26.7
71	26.6	26.5	26.6	26.8	26.7	26.9	26.6	26.7
72	26.7	26.8	27.0	27.0	27.0	27.2	27.0	27.1
73	26.7	26.8	27.0	27.1	27.1	27.2	27.2	27.2
76	26.6	26.7	26.9	26.9	27.0	27.2	26.9	27.0
77	26.6	26.7	26.9	26.9	27.0	27.2	26.9	27.0
78	26.6	26.7	26.9	26.9	27.0	27.1	26.9	26.9
79	26.6	26.7	26.9	26.8	27.0	27.2	26.9	27.0
80	26.7	26.7	26.9	26.8	27.1	27.2	27.0	27.1
83	26.5	26.6	26.7	26.7	26.8	27.0	26.8	26.9

	Nominal concentration Dienogest [ng/L]							
84	26.6	26.7	26.8	26.8	27.0	27.1	26.9	26.9
85	26.6	26.6	26.8	26.8	27.0	27.1	26.9	26.9
86	26.6	26.7	26.8	26.9	26.9	27.2	26.8	27.0
87	26.6	26.7	26.8	26.9	26.9	27.1	26.8	27.0
90	26.5	26.6	26.7	26.7	26.8	27.1	26.8	27.0
91	26.4	26.5	26.5	26.6	26.8	27.0	26.8	26.9
92	26.3	26.4	26.5	26.6	26.7	26.9	26.6	26.8
93	26.4	26.5	26.6	26.6	26.8	27.0	26.8	26.8
94	26.2	26.3	26.4	26.4	26.5	26.9	26.6	26.6
97	26.3	26.4	26.5	26.6	26.6	27.0	26.6	26.6
98	26.3	26.3	26.4	26.4	26.5	26.9	26.5	26.5
99	26.5	26.5	26.7	26.7	26.7	27.0	26.8	27.0
100	26.3	26.3	26.5	26.4	26.3	26.5	26.3	26.4
101	26.7	26.7	26.7	26.7	26.8	26.8	26.8	26.8
104	26.6	26.7	26.6	26.6	26.7	26.8	26.7	26.8
105	26.7	26.7	26.6	26.6	26.9	26.9	26.8	26.8
106	26.7	26.7	26.6	26.6	26.8	26.8	26.7	26.7
107	26.7	26.7	26.5	26.5	26.8	26.9	26.7	26.8
108	26.8	26.8	26.7	26.6	26.9	27.0	26.8	26.9
111	26.7	26.8	26.7	26.6	26.8	26.9	26.8	26.8
112	26.8	26.8	26.8	26.7	26.9	27.0	26.9	26.9
113	26.7	26.8	26.7	26.7	26.9	27.0	26.9	26.9
114	26.7	26.8	26.7	26.7	26.8	26.9	26.9	26.9
115	26.7	26.7	26.8	26.7	26.8	27.0	26.9	27.0
118	26.7	26.8	26.8	26.8	26.8	27.0	26.9	27.0
119	26.5	26.7	26.5	26.5	26.8	26.9	26.7	26.8
120	26.4	26.6	26.5	26.5	26.7	26.8	26.6	26.8
121	26.5	26.5	26.7	26.7	26.7	26.7	26.8	26.9
122	26.8	26.8	26.8	26.9	26.9	26.9	26.9	27.0
125	26.5	26.5	26.5	26.5	26.5	26.6	26.7	26.8
126	26.5	26.4	26.5	26.5	26.6	26.6	26.7	26.7
127	26.6	26.5	26.6	26.6	26.6	26.6	26.7	26.7

	Nominal concentration Dienogest [ng/L]							
128	26.7	26.5	26.6	26.6	26.6	26.7	26.8	26.8
129	-	-	-	-	-	-	-	-
Mean	**26.7**	**26.7**	**26.7**	**26.8**	**26.8**	**27.0**	**26.8**	**26.9**
SD	0.2	0.2	0.3	0.3	0.2	0.2	0.3	0.3
RSD	0.9	0.9	1.2	1.2	0.8	0.8	1.1	1.1
Min	26.2	26.3	26.3	26.3	26.3	26.5	26.3	26.3
Max	27.4	27.6	28.0	28.0	27.6	27.7	28.0	28.0

(*) During the initial phase of the study with Dienogest the water temperature was exceeded on single days. After technical adjustment, the temperature was in the recommended range until the end of the study.

Table 20: Water temperature [°C]; 100 and 320 ng Dienogest/L

	Nominal concentration Dienogest [ng/L]							
	100				320			
Replicate	A	B	C	D	A	B	C	D
Time of exposure [day]	Water temperature [°C]							
0	26.8	26.9	26.6	26.6	27.0	27.0	26.6	26.6
1	27.5	27.4	27.0	27.0	27.5	27.5	26.8	26.9
2	27.5	27.5	27.3	27.3	27.6(*)	27.6(*)	27.3	27.4
3	27.7(*)	27.8(*)	27.7(*)	27.7(*)	27.7(*)	27.9(*)	27.8(*)	27.8(*)
6	27.3	27.4	27.2	27.2	27.5	27.5	27.5	27.5
7	27.6(*)	27.6(*)	27.6(*)	27.6(*)	27.6(*)	27.7(*)	27.6(*)	27.7(*)
8	27.7(*)	27.7(*)	27.8(*)	27.8(*)	27.8(*)	27.9(*)	27.8(*)	27.9(*)
9	27.3	27.3	27.3	27.3	27.5	27.5	27.5	27.5
10	27.3	27.4	27.3	27.3	27.5	27.6(*)	27.6(*)	27.6(*)
13	26.9	26.9	26.7	26.7	26.9	27.0	27.0	27.0
14	26.9	26.9	26.7	26.7	26.9	26.9	26.9	26.9
15	27.0	27.0	27.0	26.9	27.0	27.0	27.0	27.0
16	26.8	26.9	26.9	26.8	27.0	27.0	27.0	27.0
17	26.8	26.9	26.7	26.7	26.9	26.9	26.9	26.9
20	26.8	26.9	26.6	26.6	26.8	26.9	26.8	26.9
21	26.8	26.9	26.8	26.8	26.9	27.0	27.0	26.9
22	26.8	26.9	26.8	26.8	26.9	27.0	26.9	26.9
23	26.8	26.9	26.7	26.8	26.9	27.0	27.0	26.9
24	27.0	27.0	26.7	26.8	27.1	27.1	27.1	27.0
27	26.7	26.7	26.6	26.6	26.8	26.8	26.8	26.7
28	26.6	26.6	26.6	26.6	26.8	26.7	26.7	26.6
29	26.8	26.8	26.6	26.6	26.8	26.9	26.9	26.7
30	26.8	26.8	26.6	26.6	26.8	26.9	26.9	26.8
31	26.9	26.9	26.7	26.7	26.8	26.9	26.9	26.8
34	26.6	26.7	26.5	26.4	26.7	26.8	26.8	26.8
35	26.6	26.6	26.6	26.4	26.7	26.8	26.8	26.8
36	26.5	26.6	26.6	26.5	26.7	26.7	26.8	26.7
37	26.5	26.6	26.6	26.4	26.7	26.7	26.8	26.8

	Nominal concentration Dienogest [ng/L]							
38	26.8	26.9	26.5	26.5	27.1	27.1	27.0	26.9
41	27.0	27.0	26.5	26.6	27.0	27.0	27.0	26.9
42	26.9	27.0	26.5	26.4	27.0	27.1	26.8	26.8
43	26.9	26.9	26.5	26.5	26.9	27.0	26.9	26.9
44	26.8	26.9	26.5	26.5	27.0	27.0	26.9	27.0
45	27.0	27.0	26.5	26.5	26.9	27.0	26.9	26.8
48	27.0	27.0	26.5	26.4	27.1	27.2	26.9	26.7
49	26.9	27.0	26.5	26.4	27.0	27.0	26.8	26.6
50	-	26.9	26.5	26.5	27.0	27.0	26.8	26.6
51	-	26.9	26.5	26.5	26.9	26.9	26.8	26.6
52	-	26.8	26.5	26.5	26.9	26.9	26.8	26.7
55	-	26.7	26.7	26.5	27.0	27.0	26.7	26.7
56	-	26.7	26.6	26.4	26.9	26.9	26.7	26.6
57	-	26.7	26.7	26.4	27.0	27.0	26.6	26.6
58	-	26.8	26.5	26.4	27.0	27.0	26.7	26.5
59	-	26.7	26.3	26.4	26.8	26.8	26.5	26.5
62	-	26.9	26.5	26.6	26.7	26.7	26.6	26.6
63	-	26.8	26.5	26.6	26.7	26.7	26.7	26.7
64	-	26.8	26.5	26.5	26.7	26.6	26.5	26.5
65	-	26.8	26.6	26.5	26.7	26.7	26.6	26.6
66	-	27.0	26.6	26.7	26.8	26.8	26.6	26.6
69	-	26.8	26.5	26.5	26.8	26.7	26.6	26.6
70	-	26.8	26.6	26.5	26.7	26.7	26.6	26.6
71	-	26.8	26.6	26.5	26.7	26.7	26.6	26.6
72	-	27.1	26.7	26.6	27.0	27.0	26.7	26.7
73	-	27.1	26.7	26.7	27.1	27.1	26.8	26.8
76	-	27.0	26.5	26.6	26.9	26.9	26.6	26.6
77	-	27.1	26.6	26.6	27.0	27.0	26.6	26.6
78	-	27.1	26.6	26.6	27.0	27.0	26.7	26.7
79	-	27.1	26.6	26.6	27.0	26.9	26.7	26.7
80	-	27.1	26.7	26.7	27.0	27.0	26.8	26.8
83	-	26.8	26.4	26.4	26.8	26.8	26.6	26.5

	Nominal concentration Dienogest [ng/L]							
84	-	27.0	26.5	26.5	26.9	26.8	26.5	26.5
85	-	27.0	26.5	26.5	26.9	26.8	26.5	26.6
86	-	27.0	26.4	26.5	26.9	26.8	26.5	26.5
87	-	27.0	26.4	26.5	26.9	26.8	26.5	26.5
90	-	26.8	26.2	26.3	26.8	26.8	26.4	26.4
91	-	26.8	26.2	26.2	26.7	26.6	26.4	26.3
92	-	26.7	26.2	26.2	26.7	26.6	26.3	26.3
93	-	26.8	26.3	26.3	26.6	26.6	26.4	26.4
94	-	26.7	26.1	26.1	26.4	26.5	26.2	26.2
97	-	26.6	26.2	26.2	26.5	26.5	26.3	26.3
98	-	26.5	26.1	26.1	26.3	26.4	26.2	26.2
99	-	26.5	26.4	26.4	26.8	26.8	26.8	26.8
100	-	26.3	26.1	26.0	26.2	26.2	26.2	26.2
101	-	26.7	26.4	26.4	26.7	26.7	26.7	26.6
104	-	26.6	26.3	26.3	26.6	26.7	26.6	26.5
105	-	26.6	26.4	26.4	26.7	26.8	26.6	26.5
106	-	26.6	26.4	26.4	26.7	26.8	26.6	26.5
107	-	26.6	26.3	26.3	26.6	26.7	26.5	26.5
108	-	26.7	26.4	26.4	26.7	26.9	26.7	26.6
111	-	26.7	26.4	26.4	26.7	26.9	26.7	26.6
112	-	26.8	26.5	26.5	26.8	26.9	26.7	26.6
113	-	26.8	26.4	26.4	26.8	26.9	26.6	26.6
114	-	26.8	26.5	26.5	26.8	26.9	26.6	26.6
115	-	26.8	26.4	26.4	26.7	26.9	26.5	26.5
118	-	26.9	26.5	26.5	26.8	27.0	26.6	26.6
119	-	26.8	26.3	26.3	26.6	26.8	26.5	26.5
120	-	26.8	26.3	26.3	26.6	26.7	26.5	26.5
121	-	26.7	26.4	26.4	26.6	26.7	26.6	26.6
122	-	26.5	26.4	26.4	26.8	26.9	26.8	26.7
125	-	26.3	26.1	26.1	26.5	26.7	26.5	26.5
126	-	26.3	26.2	26.2	26.5	26.6	26.6	26.5
127	-	26.4	26.3	26.2	26.5	26.6	26.6	26.6

	Nominal concentration Dienogest [ng/L]							
128	-	26.4	26.3	26.3	26.5	26.6	26.6	26.6
129	-	26.5	26.5	26.4	26.6	26.7	26.7	26.7
Mean	**27.0**	**26.9**	**26.6**	**26.6**	**26.9**	**26.9**	**26.8**	**26.7**
SD	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
RSD	1.2	1.0	1.2	1.2	1.1	1.1	1.1	1.2
Min	26.5	26.3	26.1	26.0	26.2	26.2	26.2	26.2
Max	27.7	27.8	27.8	27.8	27.8	27.9	27.8	27.9

(*) During the initial phase of the study with Dienogest the water temperature was exceeded on single days. After technical adjustment, the temperature was in the recommended range until the end of the study.

C.1.2 Test conditions: oxygen saturation

Table 21: O_2 saturation [% ASV]; control and 3.20 ng Dienogest/L

	Nominal concentration Dienogest [ng/L]							
	control				3.20			
Replicate	A	B	C	D	A	B	C	D
Time of exposure [day]	O_2 saturation [% ASV]							
0	102	102	102	102	106	107	107	106
2	92	91	87	89	90	90	89	91
6	94	93	92	91	92	93	91	91
9	97	95	95	94	96	96	96	96
13	95	94	95	95	95	95	95	95
16	96	95	93	93	96	96	96	96
20	97	96	96	96	97	97	96	96
23	100	101	100	100	99	99	99	99
27	95	98	99	98	97	97	97	95
30	96	92	92	93	88	91	93	92
34	94	98	93	93	94	94	94	95
38	94	97	93	93	94	94	94	94
41	87	92	93	93	94	93	93	91
45	103	107	105	102	103	101	101	101
48	103	107	103	101	102	101	100	99
52	102	105	104	105	101	100	99	99
55	102	107	107	102	104	103	103	103
58	103	104	102	100	101	101	102	102
63	101	103	103	100	102	103	103	101
65	97	104	102	100	102	105	103	102
69	102	104	105	106	107	105	104	105
72	100	101	100	101	104	103	102	102
76	100	100	101	101	100	101	103	103
79	98	99	99	99	95	96	96	95
83	96	97	98	97	98	99	99	98
86	97	97	97	97	98	98	97	97

	Nominal concentration Dienogest [ng/L]							
90	94	93	91	93	94	92	94	95
93	92	94	95	92	93	95	94	91
99	89	92	89	87	91	89	88	90
101	85	84	83	83	83	85	83	83
104	89	99	102	101	101	101	97	98
107	100	101	100	100	99	99	99	100
111	100	100	100	101	100	100	99	100
114	92	96	98	96	98	99	99	100
118	94	96	98	96	97	97	96	97
122	94	100	97	91	99	103	100	102
125	83	89	89	84	89	89	88	91
127	89	90	90	85	89	90	91	91
Mean	**96**	**98**	**97**	**96**	**97**	**97**	**97**	**97**
SD	5	5	6	6	5	5	5	5
RSD	5.4	5.6	5.7	5.9	5.5	5.3	5.3	5.1
Min	83	84	83	83	83	85	83	83
Max	103	107	107	106	107	107	107	106

Table 22: O_2 saturation [% ASV]; 10.0 and 32.0 ng Dienogest/L

	Nominal concentration Dienogest [ng/L]							
	10.0				32.0			
Replicate	A	B	C	D	A	B	C	D
Time of exposure [day]	O_2 saturation [% ASV]							
0	105	105	106	105	105	105	105	105
2	89	90	90	89	90	90	91	91
6	92	94	93	92	92	93	93	93
9	96	96	95	94	95	95	96	96
13	94	95	95	94	94	94	94	94
16	93	95	97	97	92	91	93	93
20	97	98	97	96	95	96	97	96
23	98	97	98	98	98	97	97	98
27	93	92	94	94	94	93	93	94
30	90	90	89	91	93	91	91	92
34	91	92	92	93	94	93	92	94
38	92	92	90	91	93	94	93	94
41	92	92	89	90	93	91	91	91
45	101	102	101	101	104	102	100	99
48	101	101	100	100	104	102	100	98
52	99	99	99	99	103	102	101	99
55	103	103	102	102	103	104	103	101
58	101	102	101	100	102	101	98	98
63	101	102	102	101	102	102	99	99
65	100	102	101	100	102	101	99	98
69	102	102	102	103	101	100	99	100
72	104	104	103	103	102	101	101	100
76	100	100	99	100	95	95	95	94
79	96	95	95	95	95	96	95	96
83	99	98	99	98	99	98	95	98
86	97	98	98	97	96	96	97	96
90	95	95	96	95	95	93	92	93
93	92	90	91	93	93	92	91	92

	Nominal concentration Dienogest [ng/L]							
99	87	87	88	89	88	84	87	88
101	83	83	83	85	86	85	83	83
104	97	98	101	100	100	100	100	100
107	99	99	98	97	97	97	98	98
111	101	101	101	100	97	97	96	96
114	98	97	97	98	98	98	96	97
118	95	96	95	96	97	97	97	97
122	97	100	97	103	99	95	92	94
125	88	88	88	89	87	87	84	87
127	89	90	88	87	88	87	88	88
Mean	**96**	**96**	**96**	**96**	**96**	**96**	**95**	**95**
SD	5	5	5	5	5	5	5	4
RSD	5.4	5.4	5.5	5.2	5.2	5.4	5.1	4.6
Min	83	83	83	85	86	84	83	83
Max	105	105	106	105	105	105	105	105

Table 23: O_2 saturation [% ASV]; 100 and 320 ng Dienogest/L

	Nominal concentration Dienogest [ng/L]							
	100				320			
Replicate	A	B	C	D	A	B	C	D
Time of exposure [day]	O_2 saturation [% ASV]							
0	105	103	103	104	104	103	102	102
2	90	89	91	92	91	91	90	91
6	93	93	94	95	95	94	94	94
9	96	95	95	96	96	95	95	96
13	93	93	94	94	94	94	94	94
16	90	91	91	91	93	94	94	94
20	95	95	96	97	97	97	97	98
23	97	97	98	97	97	96	97	97
27	94	94	94	93	91	94	94	94
30	92	93	92	91	91	91	91	92
34	96	94	92	92	91	90	92	92
38	93	93	92	91	92	93	92	94
41	92	93	93	92	91	90	91	91
45	103	102	98	96	95	95	94	94
48	102	100	96	96	94	93	93	93
52	-	97	96	95	95	94	95	94
55	-	103	103	101	102	103	102	101
58	-	95	94	95	95	95	95	95
63	-	100	100	100	100	100	99	96
65	-	101	100	100	101	101	100	98
69	-	102	102	102	101	101	102	101
72	-	100	100	102	99	99	100	100
76	-	96	95	96	95	94	95	96
79	-	94	94	93	95	94	93	94
83	-	99	99	98	97	97	96	97
86	-	95	95	96	96	95	95	95
90	-	93	93	94	94	94	95	93
93	-	93	94	94	93	94	94	94

	Nominal concentration Dienogest [ng/L]							
99	-	88	89	89	91	88	86	90
101	-	86	88	87	87	86	87	92
104	-	102	101	101	100	100	101	102
107	-	97	98	98	97	97	98	97
111	-	95	95	96	95	96	95	95
114	-	99	100	99	99	98	96	97
118	-	95	96	98	98	99	98	98
122	-	102	103	99	96	95	90	95
125	-	89	92	91	84	85	85	89
127	-	89	91	91	85	85	86	87
Mean	**95**	**96**	**96**	**96**	**95**	**95**	**95**	**95**
SD	5	4	4	4	4	4	4	3
RSD	4.8	4.7	4.2	4.1	4.6	4.7	4.6	3.6
Min	90	86	88	87	84	85	85	87
Max	105	103	103	104	104	103	102	102

C.1.3 Test conditions: pH values

Table 24: pH; control and 3.20 ng Dienogest/L

	Nominal concentration Dienogest [ng/L]							
	Control				3.20			
Replicate	A	B	C	D	A	B	C	D
Time of exposure [day]	pH value							
0	8.28	8.27	8.28	8.29	8.20	8.22	8.20	8.19
2	8.15	8.16	8.09	8.19	8.22	8.24	8.24	8.23
6	8.20	8.23	8.19	8.19	8.24	8.25	8.25	8.27
9	8.19	8.23	8.21	8.12	8.23	8.24	8.23	8.26
13	8.32	8.30	8.30	8.31	8.33	8.32	8.33	8.33
16	8.30	8.32	8.33	8.30	8.31	8.31	8.33	8.35
20	8.22	8.17	8.23	8.26	8.29	8.32	8.32	8.32
23	7.95	8.00	8.02	8.05	8.07	8.06	8.08	8.07
27	7.97	8.05	8.06	8.06	8.11	8.13	8.13	8.15
30	7.92	8.03	7.99	8.03	8.09	8.08	8.08	8.13
34	8.04	8.19	8.11	8.13	8.16	8.16	8.15	8.18
38	8.08	8.10	8.12	8.14	8.16	8.15	8.18	8.15
41	8.07	8.17	8.09	8.12	8.17	8.12	8.12	8.11
45	8.13	8.27	8.21	8.24	8.30	8.26	8.26	8.26
48	8.17	8.27	8.20	8.24	8.30	8.25	8.27	8.27
52	8.20	8.22	8.25	8.25	8.28	8.25	8.27	8.27
55	8.26	8.35	8.18	8.14	8.20	8.25	8.21	8.22
58	8.12	8.14	8.04	8.06	8.15	8.21	8.14	8.15
63	8.09	8.21	8.14	8.15	8.25	8.31	8.24	8.23
65	7.93	8.14	8.04	8.03	8.15	8.20	8.07	8.10
69	7.98	8.05	8.04	8.02	8.12	8.10	8.12	8.10
72	8.00	8.02	8.04	8.04	8.08	8.10	8.11	8.12
76	8.06	8.05	8.10	8.11	8.12	8.13	8.10	8.14
79	8.00	8.00	8.03	8.04	8.08	8.08	8.08	8.08
83	7.80	7.85	7.86	7.85	7.90	7.93	7.95	7.94
86	7.85	7.85	7.87	7.90	7.95	7.95	7.95	7.95

	Nominal concentration Dienogest [ng/L]							
90	8.01	7.90	7.83	7.82	7.92	7.87	7.89	7.91
93	7.86	7.89	7.88	7.84	7.89	7.88	7.83	7.88
99	7.93	8.01	7.95	7.92	7.98	8.03	7.91	8.00
101	7.95	7.94	7.89	7.85	7.90	7.87	7.87	7.89
104	7.80	7.83	7.84	7.76	7.91	7.90	7.85	7.88
107	7.82	7.80	7.78	7.78	7.80	7.82	7.78	7.80
111	7.85	7.83	7.83	7.83	7.85	7.85	7.84	7.83
114	7.81	7.87	7.97	7.88	8.00	8.03	7.99	8.03
118	7.92	7.95	7.95	7.93	8.00	8.02	8.00	7.98
122	7.87	7.95	7.91	7.85	8.00	8.07	7.95	8.05
125	7.70	7.86	7.86	7.72	7.86	7.86	7.83	7.92
127	7.75	7.80	7.85	7.82	7.86	7.83	7.85	7.92
Mean	**8.01**	**8.06**	**8.04**	**8.03**	**8.09**	**8.10**	**8.08**	**8.10**
SD	0.17	0.17	0.15	0.17	0.15	0.16	0.16	0.15
RSD	2.07	2.06	1.90	2.13	1.91	1.95	2.03	1.88
Min	7.70	7.80	7.78	7.72	7.80	7.82	7.78	7.80
Max	8.32	8.35	8.33	8.31	8.33	8.32	8.33	8.35

Table 25: pH; 10.0 and 32.0 ng Dienogest/L

	Nominal concentration Dienogest [ng/L]							
	10.0				32.0			
Replicate	A	B	C	D	A	B	C	D
Time of exposure [day]	pH value							
0	8.18	8.19	8.21	8.22	8.22	8.24	8.24	8.24
2	8.25	8.25	8.32	8.26	8.28	8.30	8.29	8.27
6	8.27	8.29	8.28	8.27	8.29	8.33	8.26	8.28
9	8.22	8.24	8.23	8.26	8.26	8.27	8.24	8.28
13	8.36	8.35	8.34	8.35	8.35	8.40	8.39	8.40
16	8.32	8.33	8.30	8.32	8.35	8.38	8.40	8.40
20	8.34	8.33	8.34	8.31	8.33	8.35	8.35	8.33
23	8.05	8.06	8.09	8.09	8.11	8.10	8.12	8.13
27	8.11	8.12	8.16	8.15	8.16	8.17	8.20	8.19
30	8.07	8.09	8.08	8.11	8.17	8.11	8.10	8.11
34	8.10	8.15	8.15	8.16	8.20	8.18	8.19	8.17
38	8.12	8.12	8.15	8.15	8.20	8.21	8.20	8.21
41	8.11	8.12	8.11	8.13	8.22	8.17	8.15	8.15
45	8.23	8.27	8.27	8.27	8.35	8.29	8.29	8.31
48	8.25	8.27	8.26	8.27	8.32	8.29	8.31	8.31
52	8.25	8.27	8.30	8.30	8.30	8.28	8.28	8.30
55	8.22	8.26	8.26	8.25	8.28	8.39	8.30	8.29
58	8.15	8.19	8.19	8.18	8.30	8.24	8.21	8.24
63	8.22	8.28	8.26	8.24	8.34	8.31	8.26	8.27
65	8.05	8.16	8.12	8.07	8.21	8.16	8.12	8.13
69	8.15	8.10	8.12	8.12	8.18	8.15	8.12	8.13
72	8.10	8.12	8.12	8.12	8.05	8.08	8.08	8.05
76	8.12	8.12	8.13	8.13	8.10	8.12	8.14	8.10
79	8.12	8.13	8.10	8.10	8.08	8.08	8.10	8.12
83	7.92	7.92	7.97	7.93	7.94	7.99	7.92	7.98
86	7.92	7.93	7.94	7.94	7.94	7.93	7.92	7.92
90	7.90	7.87	7.86	7.90	7.85	7.89	7.86	7.92
93	7.90	7.82	7.88	7.87	7.83	7.85	7.80	7.86

	Nominal concentration Dienogest [ng/L]							
99	7.92	7.91	7.93	7.94	7.94	7.94	7.93	7.94
101	7.85	7.86	7.87	7.88	7.98	7.98	7.90	7.90
104	7.85	7.85	7.88	7.90	7.90	7.90	7.87	7.92
107	7.82	7.82	7.80	7.80	7.80	7.78	7.80	7.79
111	7.80	7.80	7.82	7.80	7.80	7.78	7.80	7.78
114	7.92	7.94	7.96	8.01	7.99	7.96	7.97	8.02
118	7.95	7.98	8.01	8.01	7.96	7.97	7.98	8.02
122	7.92	8.01	7.91	8.07	8.02	7.92	7.91	7.89
125	7.84	7.83	7.80	7.85	7.79	7.79	7.75	7.81
127	7.82	7.82	7.81	7.83	7.80	7.82	7.77	7.80
Mean	**8.07**	**8.08**	**8.09**	**8.09**	**8.11**	**8.11**	**8.09**	**8.10**
SD	0.17	0.17	0.17	0.16	0.19	0.19	0.19	0.18
RSD	2.06	2.16	2.14	2.03	2.31	2.34	2.38	2.27
Min	7.80	7.80	7.80	7.80	7.79	7.78	7.75	7.78
Max	8.36	8.35	8.34	8.35	8.35	8.40	8.40	8.40

Table 26: pH; 100 and 320 ng Dienogest/L

	Nominal concentration Dienogest [ng/L]							
	100				320			
Replicate	A	B	C	D	A	B	C	D
Time of exposure [day]	pH value							
0	8.20	8.22	8.27	8.25	8.23	8.24	8.29	8.29
2	8.27	8.25	8.32	8.30	8.29	8.28	8.27	8.29
6	8.30	8.32	8.33	8.33	8.32	8.31	8.30	8.32
9	8.27	8.25	8.29	8.29	8.29	8.25	8.26	8.29
13	8.38	8.38	8.42	8.40	8.36	8.35	8.35	8.37
16	8.37	8.39	8.40	8.40	8.42	8.41	8.39	8.42
20	8.29	8.32	8.36	8.34	8.34	8.35	8.36	8.37
23	8.07	8.07	8.12	8.17	8.12	8.13	8.14	8.16
27	8.18	8.15	8.19	8.22	8.16	8.20	8.23	8.24
30	8.11	8.12	8.17	8.16	8.12	8.13	8.17	8.17
34	8.25	8.19	8.20	8.22	8.19	8.19	8.24	8.26
38	8.23	8.19	8.18	8.18	8.20	8.19	8.21	8.19
41	8.21	8.16	8.20	8.20	8.17	8.14	8.17	8.20
45	8.48	8.31	8.26	8.28	8.24	8.24	8.23	8.25
48	8.40	8.35	8.26	8.28	8.25	8.25	8.23	8.25
52	-	8.35	8.33	8.25	8.30	8.28	8.32	8.32
55	-	8.31	8.31	8.31	8.34	8.30	8.30	8.31
58	-	8.40	8.39	8.38	8.42	8.39	8.39	8.40
63	-	8.34	8.34	8.32	8.36	8.34	8.31	8.35
65	-	8.21	8.20	8.19	8.25	8.21	8.17	8.21
69	-	8.20	8.20	8.19	8.17	8.18	8.19	8.18
72	-	8.10	8.10	8.12	8.12	8.11	8.10	8.12
76	-	8.08	8.12	8.12	8.11	8.12	8.13	8.14
79	-	8.12	8.12	8.12	8.07	8.08	8.09	8.10
83	-	8.04	8.06	8.06	8.01	7.98	7.98	8.04
86	-	7.99	8.00	7.97	7.99	7.93	7.95	7.97
90	-	7.87	7.97	7.99	7.94	7.96	7.96	7.97
93	-	7.84	8.02	8.01	7.92	7.94	7.95	7.96

	Nominal concentration Dienogest [ng/L]							
99	-	7.94	8.01	7.99	8.02	8.00	7.94	8.02
101	-	7.93	7.98	7.97	7.95	7.92	7.91	7.93
104	-	7.80	7.85	7.85	7.87	7.85	7.88	7.90
107	-	7.75	7.75	7.75	7.78	7.75	7.77	7.75
111	-	7.75	7.77	7.79	7.80	7.82	7.83	7.84
114	-	8.08	8.13	8.17	8.11	8.03	8.00	8.09
118	-	8.10	8.12	8.13	8.15	8.10	8.02	8.05
122	-	8.10	8.14	8.08	7.98	7.97	7.92	8.06
125	-	7.86	7.96	7.94	7.77	7.81	7.79	7.85
127	-	7.85	7.95	7.98	7.80	7.82	7.80	7.80
Mean	**8.27**	**8.12**	**8.15**	**8.15**	**8.13**	**8.12**	**8.12**	**8.14**
SD	0.11	0.19	0.17	0.17	0.18	0.18	0.18	0.18
RSD	1.33	2.35	2.08	2.04	2.28	2.23	2.27	2.21
Min	8.07	7.75	7.75	7.75	7.77	7.75	7.77	7.75
Max	8.48	8.40	8.42	8.40	8.42	8.41	8.39	8.42

C.1.4 Parental generation (F_0), reproduction: fecundity and fertility

Table 27: F_0 generation, reproduction: fecundity and fertilisation rate; control

	Control																			
Replikate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Time of exposure [day]	Fertilised eggs [n]				Total egg number [n]				Fertilisation rate [%]				Female number [n]				Total egg number per female [n]			
1	58	183	138	228	60	197	147	236	96.7	92.9	93.9	96.6	5	5	5	5	12	39	29	47
2	536	438	399	145	576	502	433	148	93.1	87.3	92.1	98.0	5	5	5	5	115	100	87	30
3	388	384	282	267	407	438	337	289	95.3	87.7	83.7	92.4	5	5	5	5	81	88	67	58
4	210	0	435	73	212	0	469	74	99.1	-	92.8	98.6	5	5	5	5	42	0	94	15
5	221	420	284	0	266	473	340	0	83.1	88.8	83.5	-	5	5	5	5	53	95	68	0
6	211	40	277	164	215	41	290	171	98.1	97.6	95.5	95.9	5	5	5	5	43	8	58	34
7	470	837	352	701	512	932	400	739	91.8	89.8	88.0	94.9	5	5	5	5	102	186	80	148
8	204	488	144	344	236	628	192	367	86.4	77.7	75.0	93.7	5	5	5	5	47	126	38	73
9	438	758	267	415	494	870	331	443	88.7	87.1	80.7	93.7	5	5	5	5	99	174	66	89
10	384	282	379	290	420	320	416	302	91.4	88.1	91.1	96.0	5	5	5	5	84	64	83	60
11	244	128	162	242	256	139	209	271	95.3	92.1	77.5	89.3	5	5	5	5	51	28	42	54
12	163	436	213	260	168	519	269	272	97.0	84.0	79.2	95.6	5	5	5	5	34	104	54	54
13	362	527	275	228	387	624	317	232	93.5	84.5	86.8	98.3	5	5	5	5	77	125	63	46
14	149	493	443	401	177	538	477	464	84.2	91.6	92.9	86.4	5	5	5	5	35	108	95	93

	Control																			
15	289	474	122	475	310	544	126	487	93.2	87.1	96.8	97.5	5	5	5	5	62	109	25	97
16	112	472	223	370	116	518	274	409	96.6	91.1	81.4	90.5	5	5	5	5	23	104	55	82
17	241	153	168	68	271	178	201	76	88.9	86.0	83.6	89.5	5	5	5	5	54	36	40	15
18	213	112	145	350	219	118	156	363	97.3	94.9	92.9	96.4	5	5	5	5	44	24	31	73
19	297	363	233	257	305	402	262	301	97.4	90.3	88.9	85.4	5	5	5	5	61	80	52	60
20	0	246	338	302	0	282	381	312		87.2	88.7	96.8	5	5	5	5	0	56	76	62
21	505	167	189	199	534	186	232	211	94.6	89.8	81.5	94.3	5	5	5	5	107	37	46	42
Mean	**-**	**-**			**292**	**402**	**298**	**294**	**93.1**	**88.8**	**87.0**	**94.0**	**-**	**-**	**-**	**-**	**58**	**80**	**60**	**59**
SD	-	-	-	-	156	253	106	165	4.7	4.3	6.3	3.9	-	-	-	-	31	51	21	33
RSD	-	-	-	-	53.5	63.0	35.5	56.2	5.0	4.8	7.3	4.2	-	-	-	-	53.5	63.0	35.5	56.2
Cumulative egg number [n]					**6141**	**8449**	**6259**	**6167**												

Table 28: **F_0 generation, reproduction: fecundity and fertilisation rate; 3.20 ng Dienogest/L (nominal), respective 3.51 ng Dienogest/L (mean measured)**

	Nominal concentration: 3.20 ng Dienogest/L Mean measured concentration: 3.51 ng Dienogest/L																			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Time of exposure [day]	Fertilised eggs [n]				Total egg number [n]				Fertilisation rate [%]				Female number [n]				Total egg number per female [n]			
1	298	368	69	235	331	385	70	250	90.0	95.6	98.6	94.0	4	6	5	6	83	64	14	42
2	286	296	419	96	321	312	442	99	89.1	94.9	94.8	97.0	4	6	5	6	80	52	88	17
3	352	460	256	324	403	497	278	349	87.3	92.6	92.1	92.8	4	6	5	6	101	83	56	58
4	214	1	157	0	223	1	164	0	96.0		95.7		4	6	5	6	56	0	33	0
5	25	329	117	173	27	388	139	212	92.6	84.8	84.2	81.6	4	6	5	6	7	65	28	35
6	121	303	528	127	123	315	537	134	98.4	96.2	98.3	94.8	4	6	5	6	31	53	107	22
7	436	415	348	296	487	433	366	334	89.5	95.8	95.1	88.6	4	6	5	6	122	72	73	56
8	509	316	449	80	570	374	480	90	89.3	84.5	93.5	88.9	4	6	5	6	143	62	96	15
9	301	408	216	225	343	446	230	244	87.8	91.5	93.9	92.2	4	6	5	6	86	74	46	41
10	477	172	491	198	509	193	508	217	93.7	89.1	96.7	91.2	4	6	5	6	127	32	102	36
11	342	413	340	6	375	424	368	6	91.2	97.4	92.4	100.0	4	6	5	6	94	71	74	1
12	169	350	135	243	289	430	151	327	58.5	81.4	89.4	74.3	4	6	5	6	72	72	30	55
13	357	376	315	249	378	428	328	263	94.4	87.9	96.0	94.7	4	6	5	6	95	71	66	44
14	470	466	569	269	540	517	607	297	87.0	90.1	93.7	90.6	4	6	5	6	135	86	121	50
15	360	299	329	95	402	329	343	119	89.6	90.9	95.9	79.8	4	6	5	6	101	55	69	20

	Nominal concentration: 3.20 ng Dienogest/L **Mean measured concentration: 3.51 ng Dienogest/L**																			
16	361	466	387	303	397	493	409	342	90.9	94.5	94.6	88.6	4	6	5	6	99	82	82	57
17	276	373	450	171	307	400	473	184	89.9	93.3	95.1	92.9	4	6	5	6	77	67	95	31
18	393	154	0	154	445	167	0	167	88.3	92.2		92.2	4	6	5	6	111	28	0	28
19	315	607	657	178	330	628	689	189	95.5	96.7	95.4	94.2	4	6	5	6	83	105	138	32
20	229	130	362	0	257	138	378	0	89.1	94.2	95.8		4	6	5	6	64	23	76	0
21	412	149	706	409	475	163	733	432	86.7	91.4	96.3	94.7	4	6	5	6	119	27	147	72
Mean	**-**	**-**	**-**	**-**	**359**	**355**	**366**	**203**	**89.3**	**91.7**	**94.4**	**90.7**	**-**	**-**	**-**	**-**	**90**	**59**	**73**	**34**
SD	-	-	-	-	132	150	196	123	7.7	4.4	3.2	6.2	-	-	-	-	33	25	39	20
RSD	-	-	-	-	36.8	42.2	53.5	60.5	8.7	4.8	3.4	6.8	-	-	-	-	36.8	42.2	53.5	60.5
Cumulative egg number [n]					**7532**	**7461**	**7693**	**4255**												

Table 29: F_0 generation, reproduction: fecundity and fertilisation rate; 10.0 ng Dienogest/L (nominal), respective 10.3 ng Dienogest/L (mean measured)

	Nominal concentration: 10.0 ng Dienogest/ L Mean measured concentration: 10.3 ng Dienogest/L																			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Time of exposure [day]	**Fertilised eggs [n]**				**Total egg number [n]**				**Fertilisation rate [%]**				**Female number [n]**				**Total egg number per female [n]**			
1	19	54	288	231	20	58	309	244	95.0	93.1	93.2	94.7	4	5	6	5	5	12	52	49
2	382	664	189	195	405	752	228	209	94.3	88.3	82.9	93.3	4	5	6	5	101	150	38	42
3	322	355	732	175	343	368	774	188	93.9	96.5	94.6	93.1	4	5	6	5	86	74	129	38
4	2	253	547	153	2	294	636	161		86.1	86.0	95.0	4	5	6	5	1	59	106	32
5	0	257	646	431	0	321	724	465		80.1	89.2	92.7	4	5	6	5	0	64	121	93
6	408	180	295	303	432	186	310	312	94.4	96.8	95.2	97.1	4	5	6	5	108	37	52	62
7	524	640	659	296	555	665	728	327	94.4	96.2	90.5	90.5	4	5	6	5	139	133	121	65
8	696	513	164	256	748	560	203	332	93.0	91.6	80.8	77.1	4	5	6	5	187	112	34	66
9	300	585	357	175	325	671	401	220	92.3	87.2	89.0	79.5	4	5	6	5	81	134	67	44
10	348	324	451	259	367	342	502	287	94.8	94.7	89.8	90.2	4	5	6	5	92	68	84	57
11	0	168	56	158	1	172	58	200		97.7	96.6	79.0	4	5	6	5	0	34	10	40
12	376	425	570	339	407	460	658	356	92.4	92.4	86.6	95.2	4	5	6	5	102	92	110	71
13	409	492	281	280	428	577	289	329	95.6	85.3	97.2	85.1	4	5	6	5	107	115	48	66
14	283	266	141	301	317	287	154	330	89.3	92.7	91.6	91.2	4	5	6	5	79	57	26	66
15	513	430	561	317	546	491	636	359	94.0	87.6	88.2	88.3	4	5	6	5	137	98	106	72

	Nominal concentration: **10.0 ng Dienogest/ L** **Mean measured concentration:** **10.3 ng Dienogest/L**																			
16	396	252	98	558	437	263	114	642	90.6	95.8	86.0	86.9	4	5	6	5	109	53	19	128
17	286	550	371	0	305	593	438	0	93.8	92.7	84.7		4	5	6	5	76	119	73	0
18	155	106	414	403	172	115	456	442	90.1	92.2	90.8	91.2	4	5	6	5	43	23	76	88
19	555	585	347	146	578	648	366	157	96.0	90.3	94.8	93.0	4	5	6	5	145	130	61	31
20	7	281	319	229	23	309	348	259	30.4	90.9	91.7	88.4	4	5	6	5	6	62	58	52
21	289	664	269	212	307	738	301	264	94.1	90.0	89.4	80.3	4	5	6	5	77	148	50	53
Mean	**-**	**-**	**-**	**-**	**320**	**422**	**411**	**290**	**89.9**	**91.3**	**89.9**	**89.1**	**-**	**-**	**-**	**-**	**80**	**84**	**69**	**58**
SD	-	-	-	-	214	212	214	131	15.0	4.5	4.4	6.0	-	-	-	-	54	42	36	26
RSD	-	-	-	-	66.9	50.1	52.0	45.4	16.6	4.9	4.9	6.7	-	-	-	-	66.9	50.1	52.0	45.4
Cumulative egg number [n]					**6718**	**8870**	**8633**	**6083**												

Table 30: F_0 generation, reproduction: fecundity and fertilisation rate; 32.0 ng Dienogest/L (nominal), respective 31.7 ng Dienogest/L (mean measured)

	Nominal concentration: 32.0 ng Dienogest/L Mean measured concentration: 31.7 ng Dienogest/L																			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Time of exposure [day]	**Fertilised eggs [n]**				**Total egg number [n]**				**Fertilisation rate [%]**				**Female number [n]**				**Total egg number per female [n]**			
1	164	301	244	443	181	308	271	514	90.6	97.7	90.0	86.2	5	5	5	4	36	62	54	129
2	241	342	404	424	246	360	473	503	98.0	95.0	85.4	84.3	5	5	5	4	49	72	95	126
3	179	471	405	410	191	492	550	499	93.7	95.7	73.6	82.2	5	5	5	4	38	98	110	125
4	307	139	344	230	319	151	385	241	96.2	92.1	89.4	95.4	5	5	5	4	64	30	77	60
5	247	0	260	403	261	0	285	460	94.6	-	91.2	87.6	5	5	5	4	52	0	57	115
6	500	300	743	141	509	315	783	146	98.2	95.2	94.9	96.6	5	5	5	4	102	63	157	37
7	304	806	547	525	321	855	585	555	94.7	94.3	93.5	94.6	5	5	5	4	64	171	117	139
8	594	527	408	150	650	577	506	161	91.4	91.3	80.6	93.2	5	5	5	4	130	115	101	40
9	277	488	377	273	291	510	435	280	95.2	95.7	86.7	97.5	5	5	5	4	58	102	87	70
10	387	395	629	127	394	428	695	167	98.2	92.3	90.5	76.0	5	5	5	4	79	86	139	42
11	539	334	299	299	559	348	363	307	96.4	96.0	82.4	97.4	5	5	5	4	112	70	73	77
12	246	182	309	68	261	193	404	102	94.3	94.3	76.5	66.7	5	5	5	4	52	39	81	26
13	551	699	495	567	585	742	545	600	94.2	94.2	90.8	94.5	5	5	5	4	117	148	109	150
14	497	305	565	101	534	334	614	123	93.1	91.3	92.0	82.1	5	5	5	4	107	67	123	31
15	402	253	456	319	433	269	522	329	92.8	94.1	87.4	97.0	5	5	5	4	87	54	104	82

	Nominal concentration: 32.0 ng Dienogest/L **Mean measured concentration: 31.7 ng Dienogest/L**																			
16	626	204	480	414	691	218	580	433	90.6	93.6	82.8	95.6	5	5	5	4	138	44	116	108
17	143	119	468	258	160	129	512	265	89.4	92.2	91.4	97.4	5	5	5	4	32	26	102	66
18	416	266	334	386	443	279	393	410	93.9	95.3	85.0	94.1	5	5	5	4	89	56	79	103
19	482	190	514	270	500	196	552	278	96.4	96.9	93.1	97.1	5	5	5	4	100	39	110	70
20	201	211	325	441	211	233	352	464	95.3	90.6	92.3	95.0	5	5	5	4	42	47	70	116
21	507	327	564	439	535	357	664	500	94.8	91.6	84.9	87.8	5	5	5	4	107	71	133	125
Mean	**-**	**-**	**-**	**-**	**394**	**347**	**499**	**349**	**94.4**	**94.0**	**87.4**	**90.4**	**-**	**-**	**-**	**-**	**79**	**69**	**100**	**87**
SD	-	-	-	-	164	202	134	157	2.5	2.0	5.7	8.3	-	-	-	-	33	40	27	39
RSD	-	-	-	-	41.7	58.2	26.8	44.8	2.6	2.2	6.5	9.2	-	-	-	-	41.7	58.2	26.8	44.8
Cumulative egg number [n]					**8275**	**7294**	**10469**	**7337**												

Table 31: F_0 generation, reproduction: fecundity and fertilisation rate; 100 ng Dienogest/L (nominal), respective 105 ng Dienogest/L (mean measured)

	Nominal concentration: 100 ng Dienogest/L Mean measured concentration: 105 ng Dienogest/L																			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Time of exposure [day]	**Fertilised eggs [n]**				**Total egg number [n]**				**Fertilisation rate [%]**				**Female number [n]**				**Total egg number per female [n]**			
1	133	282	192	299	141	321	199	309	94.3	87.9	96.5	96.8	5	5	5	4	28	64	40	77
2	304	81	197	280	335	91	207	302	90.7	89.0	95.2	92.7	5	5	5	4	67	18	41	76
3	387	20	222	230	403	22	237	247	96.0	90.9	93.7	93.1	5	5	5	4	81	4	47	62
4	289	397	173	213	294	416	179	223	98.3	95.4	96.6	95.5	5	5	5	4	59	83	36	56
5	100	0	270	572	101	0	272	591	99.0	-	99.3	96.8	5	5	5	4	20	0	54	148
6	411	28	260	220	421	29	266	231	97.6	96.6	97.7	95.2	5	5	5	4	84	6	53	58
7	383	409	574	402	410	447	597	417	93.4	91.5	96.1	96.4	5	5	5	4	82	89	119	104
8	501	432	235	296	565	493	246	314	88.7	87.6	95.5	94.3	5	5	5	4	113	99	49	79
9	369	418	342	239	374	484	357	254	98.7	86.4	95.8	94.1	5	5	5	4	75	97	71	64
10	221	157	196	397	235	167	202	427	94.0	94.0	97.0	93.0	5	5	5	4	47	33	40	107
11	291	42	230	21	305	42	243	23	95.4	100	94.7	91.3	5	5	5	4	61	8	49	6
12	349	158	203	498	362	184	210	525	96.4	85.9	96.7	94.9	5	5	5	4	72	37	42	131
13	556	261	7	118	595	302	9	125	93.4	86.4	77.8	94.4	5	5	5	4	119	60	2	31
14	199	137	80	495	213	168	83	522	93.4	81.5	96.4	94.8	5	5	5	4	43	34	17	131
15	215	200	1103	129	226	234	1123	137	95.1	85.5	98.2	94.2	5	5	5	4	45	47	225	34

	Nominal concentration: 100 ng Dienogest/L **Mean measured concentration: 105 ng Dienogest/L**																			
16	249	231	383	351	260	270	412	369	95.8	85.6	93.0	95.1	5	5	5	4	52	54	82	92
17	286	520	0	237	293	572	0	256	97.6	90.9		92.6	5	5	5	4	59	114	0	64
18	312	117	19	279	331	120	20	318	94.3	97.5	95.0	87.7	5	5	5	4	66	24	4	80
19	98	323	816	218	110	351	840	225	89.1	92.0	97.1	96.9	5	5	5	4	22	70	168	56
20	629	261	159	533	727	271	164	566	86.5	96.3	97.0	94.2	5	5	5	4	145	54	33	142
21	288	335	379	86	305	355	387	93	94.4	94.4	97.9	92.5	5	5	5	4	61	71	77	23
Mean	**-**	**-**	**-**	**-**	**334**	**254**	**298**	**308**	**94.4**	**90.8**	**95.4**	**94.1**	**-**	**-**	**-**	**-**	**67**	**51**	**60**	**77**
SD	-	-	-	-	156	170	270	156	3.4	5.0	4.4	2.1	-	-	-	-	31	34	54	39
RSD	-	-	-	-	46.7	67.0	90.8	50.7	3.6	5.5	4.6	2.3	-	-	-	-	46.7	67.0	90.8	50.7
Cumulative egg number [n]					**7006**	**5339**	**6253**	**6474**												

Table 32: F_0 generation, reproduction: fecundity and fertilisation rate 320 ng Dienogest/L (nominal), respective 335 ng Dienogest/L (mean measured)

	Nominal concentration: Mean measured concentration:				320 ng Dienogest/L 335 ng Dienogest/L															
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Time of exposure [day]	Fertilised eggs [n]				Total egg number [n]				Fertilisation rate [%]				Female number [n]				Total egg number per female [n]			
1	371	473	267	388	379	551	285	438	97.9	85.8	93.7	88.6	5	4	5	5	76	138	57	88
2	85	107	267	307	92	122	297	379	92.4	87.7	89.9	81.0	5	4	5	5	18	31	59	76
3	586	462	353	618	646	513	384	757	90.7	90.1	91.9	81.6	5	4	5	5	129	128	77	151
4	104	117	257	122	112	119	305	153	92.9	98.3	84.3	79.7	5	4	5	5	22	30	61	31
5	210	0	193	609	216	0	221	690	97.2		87.3	88.3	5	4	5	5	43	0	44	138
6	249	139	173	493	279	147	198	516	89.2	94.6	87.4	95.5	5	4	5	5	56	37	40	103
7	789	556	357	756	913	572	407	845	86.4	97.2	87.7	89.5	5	4	5	5	183	143	81	169
8	498	127	433	270	561	128	452	353	88.8	99.2	95.8	76.5	5	4	5	5	112	32	90	71
9	330	242	447	422	390	252	472	521	84.6	96.0	94.7	81.0	5	4	5	5	78	63	94	104
10	371	135	490	410	426	137	528	494	87.1	98.5	92.8	83.0	5	4	5	5	85	34	106	99
11	343	390	43	364	355	399	43	407	96.6	97.7	100.0	89.4	5	4	5	5	71	100	9	81
12	249	179	82	614	269	189	90	704	92.6	94.7	91.1	87.2	5	4	5	5	54	47	18	141
13	365	248	342	274	426	256	403	317	85.7	96.9	84.9	86.4	5	4	5	5	85	64	81	63
14	504	199	864	472	637	204	877	578	79.1	97.5	98.5	81.7	5	4	5	5	127	51	175	116
15	405	89	176	433	477	92	188	495	84.9	96.7	93.6	87.5	5	4	5	5	95	23	38	99

	Nominal concentration: **Mean measured concentration:**				**320 ng Dienogest/L** **335 ng Dienogest/L**															
16	384	351	414	772	460	365	488	928	83.5	96.2	84.8	83.2	5	4	5	5	92	91	98	186
17	22	0	343	193	26	0	366	232	84.6		93.7	83.2	5	4	5	5	5	0	73	46
18	597	360	237	145	694	385	250	156	86.0	93.5	94.8	92.9	5	4	5	5	139	96	50	31
19	451	244	339	579	505	261	366	661	89.3	93.5	92.6	87.6	5	4	5	5	101	65	73	132
20	247	140	105	272	290	149	122	307	85.2	94.0	86.1	88.6	5	4	5	5	58	37	24	61
21	399	158	342	205	471	168	380	219	84.7	94.0	90.0	93.6	5	4	5	5	94	42	76	44
Mean	**-**	**-**	**-**	**-**	**411**	**239**	**339**	**483**	**88.5**	**94.9**	**91.2**	**86.0**	**-**	**-**	**-**	**-**	**82**	**60**	**68**	**97**
SD	-	-	-	-	214	167	181	222	4.9	3.6	4.5	4.9	-	-	-	-	43	42	36	44
RSD	-	-	-	-	52.1	70.1	53.5	45.8	5.6	3.8	4.9	5.7	-	-	-	-	52.1	70.1	53.5	45.8
Cumulative egg number [n]					**8624**	**5009**	**7122**	**10150**												

Table 33: F_0 generation, summary: reproduction

		Nominal concentration Dienogest [ng/L]					
		Control	**3.20**	**10.0**	**32.0**	**100**	**320**
		Mean measured concentration Dienogest [ng/L]					
	Replicate	**Control**	**3.51**	**10.3**	**31.7**	**105**	**335**
Total eggs per day and female [n]	A	58	90	80	79	67	82
	B	80	59	84	69	51	60
	C	60	73	69	100	60	68
	D	59	34	58	87	77	97
	Mean	**64**	**64**	**73**	**84**	**64**	**77**
	SD	11	24	12	13	11	16
	RSD	16.7	36.8	16.2	15.3	17.5	21.3
Fertilisation rate [%]	A	93.1	89.3	89.9	94.4	94.4	88.5
	B	88.8	91.7	91.3	94.0	90.8	94.9
	C	87.0	94.4	89.9	87.4	95.4	91.2
	D	94.0	90.7	89.1	90.4	94.1	86.0
	Mean	**90.7**	**91.5**	**90.1**	**91.5**	**93.7**	**90.2**
	SD	3.4	2.2	0.9	3.3	2.0	3.8
	RSD	3.7	2.4	1.0	3.6	2.1	4.2

C.1.5 Parental generation (F_0): total length and wet weight at termination

Table 34: F_0 generation, individual total length at termination [cm]; control and 3.20 ng Dienogest/L (nominal), respective 3.51 ng Dienogest/L (mean measured)

	Control								Nominal concentration: 3.20 ng Dienogest/L Mean measured concentration: 3.51 ng Dienogest/L							
	Total length [cm]															
Replicate	A		B		C		D		A		B		C		D	
Fish No.	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	-	3.5	3.8	-	-	4.4	3.8	-	-	3.8	4.1	-	-	4.1	-	4.4
2	3.8	-	-	4.2	3.8	-	-	4.4	-	4.2	-	3.9	4.0	-	-	4.3
3	-	4.3	4.0	-	-	3.6	3.8	-	-	4.0	-	4.0	-	4.0	-	4.1
4	4.0	-	3.9	-	-	4.0	4.0	-	3.5	-	3.8	-	-	3.9	3.8	-
5	3.8	-	-	4.2	4.0	-	-	4.5	3.8	-	-	3.9	-	3.9	-	3.9
6	-	3.8	-	3.6	-	3.8	-	3.9	4.0	-	3.8	-	3.7	-	-	4.1
7	3.9	-	3.9	-	3.8	-	-	3.8	3.9	-	-	3.8	-	3.8	3.7	-
8	-	3.5	-	3.9	-	3.6	-	4.0	-	3.4	-	3.2	3.9	-	3.8	-
9	4.2	-	3.8	-	3.6	-	3.8	-	4.0	-	3.5	-	3.6	-	3.6	-
10	-	3.7	-	3.6	-	-	3.8	-	3.7	-	-	3.5	3.8	-	-	3.5
Mean	**3.9**	**3.8**	**3.9**	**3.9**	**3.8**	**3.9**	**3.8**	**4.1**	**3.8**	**3.9**	**3.8**	**3.7**	**3.8**	**3.9**	**3.7**	**4.1**
SD	0.2	0.3	0.1	0.3	0.2	0.3	0.1	0.3	0.2	0.3	0.2	0.3	0.2	0.1	0.1	0.3
RSD	4.2	8.7	2.2	7.7	4.3	8.6	2.3	7.6	5.1	8.9	6.4	8.2	4.2	2.9	2.6	7.9

Table 35: F_0 generation, individual total length at termination [cm]; 10.0 and 32.0 ng Dienogest/L (nominal), respective 10.3 and 31.7 ng Dienogest/L (mean measured)

	Nominal concentration: 10.0 ng Dienogest/L Mean measured concentration: 10.3 ng Dienogest/L								Nominal concentration: 32.0 ng Dienogest/L Mean measured concentration: 31.7 ng Dienogest/L							
	Total length [cm]															
Replicate	**A**		**B**		**C**		**D**		**A**		**B**		**C**		**D**	
Fish No.	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
1	-	4.8(*)	3.6	-	-	4.5	4.0	-	-	4.1	4.0	-	-	3.8	-	4.0
2	-	4.0	4.1	-	4.0	-	-	4.0	-	3.9	-	3.8	-	4.0	4.0	-
3	-	3.5	-	3.8	-	3.6	4.1	-	-	4.0	4.0	-	-	4.2	-	4.1
4	3.9		-	4.0	-	3.9	4.0	-	-	4.0	-	3.7	4.0	-	-	4.4
5	-	4.0	-	4.3	3.8	-	3.8	-	3.8	-	3.5	-	-	4.0	4.0	-
6	-	3.8	-	3.8	-	3.8	3.8	-	3.9	-	-	4.5	3.8	-	-	4.0
7	3.8	-	-	4.0	3.4	-	-	3.3	-	3.7	-	4.0	4.0	-	3.5	-
8	4.0	-	3.8	-	-	3.9	-	4.6	4.0	-	4.0	-	-	3.6	3.6	-
9	3.8	-	3.8	-	-	3.9	-	3.5	3.6	-	3.8	-	3.5	-	4.0	-
10	4.0	-	3.6	-	3.8	-	-	3.8	3.6	-	-	4.0	3.8	-	-	-
Mean	**3.9**	**3.8**	**3.8**	**4.0**	**3.8**	**3.9**	**3.9**	**3.8**	**3.8**	**3.9**	**3.9**	**4.0**	**3.8**	**3.9**	**3.8**	**4.1**
SD	0.1	0.2	0.2	0.2	0.3	0.3	0.1	0.5	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2
RSD	2.6	6.2	5.4	5.1	6.7	7.7	3.4	13.1	4.7	3.8	5.7	7.7	5.4	5.8	6.5	4.6

(*) The hermaphrodite fish was excluded from calculation of the mean.

Table 36: F_0 generation, individual total length at termination [cm]; 100 and 320 ng Dienogest/L (nominal), respective 105 and 335 ng Dienogest/L (mean measured)

	Nominal concentration: 100 ng Dienogest/L Mean measured concentration: 105 ng Dienogest/L								Nominal concentration: 320 ng Dienogest/L Mean measured concentration: 335 ng Dienogest/L							
	Total length [cm]															
Replicate	**A**		**B**		**C**		**D**		**A**		**B**		**C**		**D**	
Fish No.	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
1	3.8	-	-	4.4	-	4.0	-	4.3	-	4.0	-	4.2	3.8	-	4.0	-
2	-	4.0	-	3.8	-	4.0	3.7	-	-	3.9	-	3.9	-	3.9	-	3.7
3	-	4.0	-	4.0	3.9	-	4.1	-	-	3.9	-	4.2	4.1	-	-	3.7
4	-	4.2	-	3.6	-	4.0	-	3.9	-	3.9	3.7	-	-	4.1	-	4.0
5	-	4.0	-	3.6	-	3.8	3.9	-	4.0	-	-	3.7	-	3.7	-	4.0
6	4.0	-	4.0	-	3.8	-	-	4.0	-	3.9	3.9	-	3.8	-	3.8	-
7	3.8	-	3.8	-	-	3.8	-	3.7	4.0	-	4.1	-	-	4.0	-	3.7
8	-	3.6	3.7	-	4.0	-	3.8	-	3.6	-	4.0	-	3.7	-	3.8	-
9	4.0	-	3.8	-	4.2	-	4.1	-	4.0	-	3.6	-	-	3.5	4.0	-
10	3.5	-	3.8	-	3.8	-	3.9	-	3.9	-	3.9	-	3.6	-	3.8	-
Mean	**3.8**	**4.0**	**3.8**	**3.9**	**3.9**	**3.9**	**3.9**	**4.0**	**3.9**	**3.9**	**3.9**	**4.0**	**3.8**	**3.8**	**3.9**	**3.8**
SD	0.2	0.2	0.1	0.3	0.2	0.1	0.2	0.3	0.2	0.0	0.2	0.2	0.2	0.2	0.1	0.2
RSD	5.4	5.5	2.9	8.6	4.2	2.8	4.1	6.3	4.4	1.1	4.8	6.1	4.9	6.3	2.8	4.3

Table 37: F_0 generation, individual wet weight at termination [g]; control and 3.20 ng Dienogest/L (nominal), respective 3.51 ng Dienogest/L (mean measured)

	Control								**Nominal concentration: 3.20 ng Dienogest/L Mean measured concentration: 3.51 ng Dienogest/L**							
	Wet weight [g]															
Replicate	**A**		**B**		**C**		**D**		**A**		**B**		**C**		**D**	
Fish No.	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
1	-	0.500	0.446	-	-	0.968	0.458	-	-	0.715	0.577	-	-	0.762	-	1.070
2	0.512	-	-	0.941	0.549	-	-	1.078	-	0.972	-	0.600	0.450	-	-	0.993
3	-	1.080	0.529	-	-	0.569	0.438	-	-	0.767	-	0.695	-	0.591	-	0.880
4	0.620	-	0.525	-	-	0.880	0.553	-	0.392	-	0.460	-	-	0.767	0.439	-
5	0.441	-	-	0.813	0.563	-	-	0.873	0.451	-	0.540	-	-	0.630	-	0.612
6	-	0.676	-	0.611	-	0.626	-	0.714	0.543	-	0.469	-	0.468	-	-	0.731
7	0.428	-	0.495	-	0.434	-	-	0.484	0.507	-	-	0.568	-	0.640	0.481	-
8	-	0.408	-	0.783	-	0.474	-	0.661	-	0.452	-	0.388	0.553	-	0.499	-
9	0.592	-	0.487	-	0.525	-	0.470	-	0.504	-	0.480	-	0.433	-	0.440	-
10	-	0.521	-	0.564	-	-	0.470	-	0.462	-	-	0.347	0.448	-	-	0.495
Mean	**0.519**	**0.637**	**0.496**	**0.742**	**0.518**	**0.703**	**0.478**	**0.762**	**0.477**	**0.727**	**0.505**	**0.520**	**0.470**	**0.678**	**0.465**	**0.797**
SD	0.087	0.266	0.034	0.154	0.058	0.211	0.044	0.225	0.053	0.214	0.051	0.147	0.048	0.081	0.030	0.223
RSD	16.7	41.7	6.8	20.8	11.2	30.0	9.2	29.5	11.1	29.5	10.1	28.3	10.2	12.0	6.5	28.0

Table 38: F_0 generation, individual wet weight at termination [g]; 10.0 and 32.0 ng Dienogest/L, respective 10.3 and 31.7 ng Dienogest/L (mean measured)

	Nominal concentration: 10.0 ng Dienogest/L Mean measured concentration: 10.3 ng Dienogest/L								**Nominal concentration: 32.0 ng Dienogest/L Mean measured concentration: 31.7 ng Dienogest/L**							
	Wet weight [g]															
Replicate	**A**		**B**		**C**		**D**		**A**		**B**		**C**		**D**	
Fish No.	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
1	-	1.180(*)	0.391	-	-	1.014	0.504	-	-	0.657	0.595	-	-	0.590	-	0.772
2	-	0.748	0.638	-	0.519	-	-	0.827	-	0.812	-	0.677	-	0.637	0.490	-
3	-	0.545	-	0.594	-	0.477	0.628	-	-	0.768	0.520	-	-	0.756	-	0.671
4	0.467	-	-	0.734	-	0.599	0.545	-	-	0.663	-	0.635	0.631	-	-	1.018
5	-	0.700	-	0.925	0.455	-	0.528	-	0.465	-	0.395	-	-	0.705	0.512	-
6	-	0.610	-	0.513	-	0.670	0.519	-	0.504	-	-	0.977	0.531	-	-	0.703
7	0.455	-	-	0.683	0.400	-	-	0.351	-	0.499	-	0.631	0.579	-	0.427	-
8	0.522	-	0.466	-	-	0.595	-	1.347	0.535	-	0.523	-	-	0.537	0.469	-
9	0.439	-	0.461	-	-	0.521	-	0.614	0.450	-	0.465	-	0.443	-	0.524	-
10	0.502	-	0.437	-	0.481	-	-	0.589	0.412	-	-	0.760	0.474	-	-	-
Mean	**0.477**	**0.651**	**0.479**	**0.690**	**0.464**	**0.646**	**0.545**	**0.746**	**0.473**	**0.680**	**0.500**	**0.736**	**0.532**	**0.645**	**0.484**	**0.791**
SD	0.034	0.091	0.094	0.156	0.050	0.192	0.049	0.376	0.048	0.121	0.075	0.144	0.076	0.088	0.038	0.157
RSD	7.2	14.0	19.6	22.7	10.8	29.8	9.0	50.4	10.1	17.8	14.9	19.6	14.4	13.6	7.9	19.9

(*) The hermaphrodite fish was excluded from calculation of the mean.

Table 39: F_0 generation, individual wet weight at termination [g]; 100 and 320 ng Dienogest/L (nominal), respective 105 and 335 ng Dienogest/L (mean measured)

	Nominal concentration: 100 ng Dienogest/L Mean measured concentration: 105 ng Dienogest/L								Nominal concentration: 320 ng Dienogest/L Mean measured concentration: 335 ng Dienogest/L							
	Wet weight [g]															
Replicate	**A**		**B**		**C**		**D**		**A**		**B**		**C**		**D**	
Fish No.	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
1	0.401	-	-	1.055	-	0.630	-	0.836	-	0.779	-	0.696	0.510	-	0.554	-
2	-	0.774	-	0.728	-	0.617	0.407	-	-	0.576	-	0.727	-	0.760	-	0.561
3	-	0.695	-	0.794	0.464	-	0.591	-	-	0.566	-	0.890	0.515	-	-	0.553
4	-	0.857	-	0.348	-	0.643	-	0.752	-	0.752	0.390	-	-	0.857	-	0.745
5	-	0.643	-	0.322	-	0.531	0.608	-	0.542	-	-	0.649	-	0.477	-	0.780
6	0.501	-	0.579	-	0.528	-	-	0.680	-	0.630	0.511	-	0.512	-	0.497	-
7	0.534	-	0.474	-	-	0.595	-	0.469	0.540	-	0.560	-	-	0.664	-	0.418
8	-	0.489	0.431	-	0.548	-	0.463	-	0.384	-	0.607	-	0.454	-	0.490	-
9	0.499	-	0.491	-	0.620	-	0.571	-	0.520	-	0.422	-	-	0.482	0.558	-
10	0.343	-	0.479	-	0.433	-	0.504	-	0.502	-	0.520	-	0.422	-	0.460	-
Mean	**0.456**	**0.692**	**0.491**	**0.649**	**0.519**	**0.603**	**0.524**	**0.684**	**0.498**	**0.661**	**0.502**	**0.741**	**0.483**	**0.648**	**0.512**	**0.611**
SD	0.080	0.139	0.054	0.312	0.073	0.044	0.079	0.157	0.066	0.099	0.082	0.105	0.042	0.168	0.043	0.150
RSD	17.6	20.1	11.1	48.1	14.2	7.3	15.2	22.9	13.2	15.0	16.4	14.1	8.8	26.0	8.3	24.5

Table 40: F_0 generation, summary: mean total length and mean wet weight at termination

		Nominal concentration Dienogest [ng/L]					
		Control	**3.20**	**10.0**	**32.0**	**100**	**320**
		Mean measured concentration Dienogest [ng/L]					
	Replicate	**Control**	**3.51**	**10.3**	**31.7**	**105**	**335**
Total length males at termination [cm]	A	3.9	3.8	3.9	3.8	3.8	3.9
	B	3.9	3.8	3.8	3.9	3.8	3.9
	C	3.8	3.8	3.8	3.8	3.9	3.8
	D	3.8	3.7	3.9	3.8	3.9	3.9
	Mean	**3.9**	**3.8**	**3.8**	**3.8**	**3.9**	**3.9**
	SD	0.1	0.0	0.1	0.0	0.1	0.0
	RSD	1.5	1.1	2.4	0.9	1.5	1.1
Total length females at termination [cm]	A	3.8	3.9	3.8	3.9	4.0	3.9
	B	3.9	3.7	4.0	4.0	3.9	4.0
	C	3.9	3.9	3.9	3.9	3.9	3.8
	D	4.1	4.1	3.8	4.1	4.0	3.8
	Mean	**3.9**	**3.9**	**3.9**	**4.0**	**3.9**	**3.9**
	SD	0.2	0.1	0.1	0.1	0.0	0.1
	RSD	3.8	3.6	1.9	2.3	1.1	2.1
Wet weight males at termination [g]	A	0.519	0.477	0.477	0.473	0.456	0.498
	B	0.496	0.505	0.479	0.500	0.491	0.502
	C	0.518	0.470	0.464	0.532	0.603	0.483
	D	0.478	0.465	0.545	0.484	0.524	0.512
	Mean	**0.503**	**0.479**	**0.491**	**0.497**	**0.518**	**0.498**
	SD	0.019	0.018	0.036	0.025	0.063	0.012
	RSD	3.9	3.8	7.4	5.1	12.2	2.4
Wet weight females at termination [g]	A	0.637	0.727	0.651	0.680	0.692	0.661
	B	0.742	0.520	0.690	0.736	0.649	0.741
	C	0.703	0.678	0.646	0.645	0.603	0.648
	D	0.762	0.797	0.746	0.791	0.684	0.611
	Mean	**0.711**	**0.680**	**0.683**	**0.713**	**0.657**	**0.665**
	SD	0.055	0.118	0.046	0.064	0.040	0.054
	RSD	7.8	17.3	6.7	9.0	6.1	8.2

Table 41: F_0 generation, sex ratio, control, 3.20 and 10.0 ng Dienogest/L (nominal), respective 3.51 and 10.3 ng Dienogest/L (mean measured); (based on histological data provided in C.1.13)

	Nominal concentration Dienogest [ng/L]											
	Control				**3.20**				**10.0**			
	Mean measured concentration Dienogest [ng/L]											
	Control				**3.51**				**10.3**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Male [n]	5	5	4	5	6	4	5	4	5	5	4	5
Female [n]	5	5	5	5	4	6	5	6	4	5	6	5
Hermaphrodite [n]	0	0	0	0	0	0	0	0	1	0	0	0
Total [n]	10	10	9	10	10	10	10	10	10	10	10	10
Male [%]	50.0	50.0	44.4	50.0	60.0	40.0	50.0	40.0	50.0	50.0	40.0	50.0
Female [%]	50.0	50.0	55.6	50.0	40.0	60.0	50.0	60.0	40.0	50.0	60.0	50.0
Hermaphrodite [%]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	0.0
Total [%]	100	100	100	100	100	100	100	100	100	100	100	100
Total mean male [%]	**48.6**				**47.5**				**47.5**			
SD	2.8				9.6				5.0			
RSD	5.7				20.2				10.5			
Total mean female [%]	**51.4**				**52.5**				**50.0**			
SD	2.8				9.6				8.2			
RSD	5.4				18.2				16.3			
Total mean hermaphrodite [%]	**0.0**				**0.0**				**2.5**			
SD	0.0				0.0				5.0			
RSD	-				-				200.0			

Table 42: F_0 generation, sex ratio, 32.0, 100 and 320 ng Dienogest/L (nominal), respective 31.7, 105 and 335 ng Dienogest/L (mean measured); (based on histological data provided in C.1.13)

	Nominal concentration Dienogest [ng/L]											
	32.0				**100**				**320**			
	Mean measured concentration Dienogest [ng/L]											
	31.7				**105**				**335**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Male [n]	5	5	5	5	5	5	5	6	5	6	5	5
Female [n]	5	5	5	4	5	5	5	4	5	4	5	5
Hermaphrodite [n]	0	0	0	0	0	0	0	0	0	0	0	0
Total [n]	10	10	10	9	10	10	10	10	10	10	10	10
Male [%]	50.0	50.0	50.0	55.6	50.0	50.0	50.0	60.0	50.0	60.0	50.0	50.0
Female [%]	50.0	50.0	50.0	44.4	50.0	50.0	50.0	40.0	50.0	40.0	50.0	50.0
Hermaphrodite [%]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total [%]	100	100	100	100	100	100	100	100	100	100	100	100
Total mean male [%]	**51.4**				**52.5**				**52.5**			
SD	2.8				5.0				5.0			
RSD	5.4				9.5				9.5			
Total mean female [%]	**48.6**				**47.5**				**47.5**			
SD	2.8				5.0				5.0			
RSD	5.7				10.5				10.5			
Total mean hermaphrodite [%]	**0.0**				**0.0**				**0.0**			
SD	0.0				0.0				0.0			
RSD	-				-				-			

C.1.6 First filial generation (F_1), early life stage

Table 43: F_1 generation, early life stage: hatching success, survival at day 21 and 35 pf

		Nominal concentration Dienogest [ng/L]					
		Control	3.20	10.0	32.0	100	320
		Mean measured concentration Dienogest [ng/L]					
	Replicate	Control	3.51	10.3	31.7	105	335
Introduced eggs [n]	A	36	36	36	36	36	36
	B	36	36	36	36	36	36
	C	36	36	36	36	36	36
	D	36	36	36	36	36	36
Hatch, day 2 pf [n]	A	0	0	0	0	0	1
	B	0	0	0	0	0	0
	C	0	0	0	0	0	0
	D	1	0	0	0	0	0
Hatch, day 3 pf [n]	A	3	5	1	7	2	1
	B	7	0	0	0	4	3
	C	8	8	4	13	4	2
	D	8	12	21	11	5	6
Hatch, day 4 pf [n]	A	35	36	35	36	34	36
	B	36	35	36	35	36	36
	C	36	36	36	36	36	36
	D	36	35	36	35	36	35
Hatch, day 5 pf [n]	A	36	36	36	36	36	36
	B	36	36	36	36	36	36
	C	36	36	36	36	36	36
	D	36	36	36	36	36	36
Hatch, day 5 pf [%]	A	100	100	100	100	100	100
	B	100	100	100	100	100	100
	C	100	100	100	100	100	100
	D	100	100	100	100	100	100
	Mean	**100**	**100**	**100**	**100**	**100**	**100**
	SD	0.0	0.0	0.0	0.0	0.0	0.0
	RSD	0.0	0.0	0.0	0.0	0.0	0.0
Survived, day 21 pf [n]	A	33	28	32	25	0	12
	B	32	30	24	31	31	9
	C	34	29	21	20	7	30
	D	35	29	25	29	17	18

		Nominal concentration Dienogest [ng/L]					
Survived, day 21 pf [%]	A	91.7	77.8	88.9	69.4	0.0	33.3
	B	88.9	83.3	66.7	86.1	86.1	25.0
	C	94.4	80.6	58.3	55.6	19.4	83.3
	D	97.2	80.6	69.4	80.6	47.2	50.0
	Mean	**93.1**	**80.6**	**70.8**	**72.9**	**38.2(*)**	**47.9(*)**
	SD	3.6	2.3	12.9	13.5	37.4	25.8
	RSD	3.9	2.8	18.3	18.5	97.8	53.8
Survived, day 35 pf [n]	A	33	28	32	24	0	12
	B	31	30	24	31	30	9
	C	34	28	21	19	7	29
	D	35	28	25	29	17	18
Survived, day 35 pf [%]	A	91.7	77.8	88.9	66.7	0.0	33.3
	B	86.1	83.3	66.7	86.1	83.3	25.0
	C	94.4	77.8	58.3	52.8	19.4	80.6
	D	97.2	77.8	69.4	80.6	47.2	50.0
	Mean	**92.4**	**79.2**	**70.8**	**71.5**	**37.5(*)**	**47.2(*)**
	SD	4.7	2.8	12.9	14.9	36.2	24.5
	RSD	5.1	3.5	18.3	20.9	96.5	52.0

(*) Statistically significant reduction compared to control, $p<0.05$, Williams test, one-sided smaller.

Table 44: F_1 generation, early life stage: individual total length at day 35 pf [cm]; control, 3.20 and 10.0 ng Dienogest/L (nominal), respective 3.51 and 10.3 ng Dienogest/L (mean measured)

	Nominal concentration Dienogest [ng/L]											
	Control				3.20				10.0			
	Mean measured concentration Dienogest [ng/L]											
	Control				3.51				10.3			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Individual total length day 35 pf [cm]											
1	1.82	1.97	2.32	2.13	1.69	1.80	1.89	1.64	1.65	1.77	1.75	2.03
2	1.96	1.96	1.71	1.83	1.82	1.23	1.93	1.70	1.81	1.91	1.66	2.02
3	2.26	1.81	2.03	1.65	1.76	1.77	1.75	1.91	1.66	1.36	1.81	1.75
4	1.56	1.91	1.90	1.83	1.53	1.93	1.39	2.24	1.67	1.72	1.64	1.94
5	1.86	1.99	1.50	2.01	1.51	1.90	1.70	1.61	1.78	1.89	1.72	1.57
6	1.82	1.85	2.06	2.04	1.78	1.68	1.77	1.67	1.80	1.85	1.89	1.76
7	1.68	1.82	1.92	2.07	1.24	1.77	1.93	2.00	1.78	1.27	2.09	1.82
8	1.70	1.93	2.03	1.65	2.10	2.01	1.97	1.77	1.88	1.74	1.80	1.44
9	2.17	2.07	2.29	2.40	1.71	1.58	2.04	1.78	1.49	1.58	2.14	1.86
10	1.89	1.76	1.95	1.47	1.84	1.94	2.13	1.72	2.05	1.89	1.86	1.72
11	1.51	1.86	2.04	1.59	1.99	2.23	2.20	1.98	1.78	1.94	1.73	2.13
12	1.87	2.06	2.19	1.74	1.97	1.83	1.75	1.98	1.86	1.30	1.63	1.56
13	2.14	1.80	1.99	1.90	1.53	1.64	1.70	1.51	1.79	1.74	1.65	1.90
14	2.02	1.92	1.90	1.67	2.09	1.76	1.54	2.08	1.50	2.04	1.76	1.88
15	1.97	1.87	1.90	2.00	1.99	1.70	1.71	1.33	1.70	2.04	1.83	1.62
16	1.79	1.99	1.48	1.74	2.01	1.55	2.09	2.09	1.58	1.68	1.72	1.72
17	1.77	2.11	1.96	1.62	1.78	1.43	1.79	1.50	1.22	1.21	1.65	1.56
18	1.82	2.01	1.61	1.78	1.75	1.96	1.62	1.80	1.55	1.75	1.87	1.77
19	1.43	1.85	1.86	1.73	2.02	1.62	1.95	2.16	1.69	2.02	1.89	1.51
20	2.04	2.16	2.08	2.13	1.74	2.09	1.86	1.50	1.79	1.87	1.72	1.98
21	1.07	2.24	2.07	1.69	1.98	1.90	1.97	1.62	1.43	2.03	1.26	1.20
22	1.06	1.78	1.75	1.84	1.54	1.86	1.68	1.57	1.81	1.99	-	2.01
23	1.60	2.00	1.90	1.60	1.76	1.17	1.34	1.36	1.43	0.59	-	1.75
24	1.72	1.94	1.80	1.85	1.99	1.52	1.63	1.49	1.82	1.80	-	1.81
25	1.42	1.76	2.07	2.00	1.17	1.82	1.75	2.33	1.70	-	-	1.89

	Nominal concentration Dienogest [ng/L]											
26	2.00	1.91	2.10	1.59	1.52	1.44	1.60	1.84	1.93	-	-	-
27	1.77	1.78	1.90	2.19	1.71	1.99	1.68	2.05	1.28	-	-	-
28	1.80	1.17	2.10	1.90	1.86	2.14	1.89	1.83	1.64	-	-	-
29	2.02	1.85	1.82	1.74	-	1.94	-	-	1.66	-	-	-
30	1.72	1.65	1.72	2.00	-	1.54	-	-	1.62	-	-	-
31	2.05	1.14	1.49	1.88	-	-	-	-	1.63	-	-	-
32	1.92	-	1.86	1.77	-	-	-	-	1.45	-	-	-
33	1.89	-	1.50	1.86	-	-	-	-	-	-	-	-
34	-	-	1.97	1.71	-	-	-	-	-	-	-	-
35	-	-	-	1.84	-	-	-	-	-	-	-	-
Mean	**1.79**	**1.87**	**1.91**	**1.84**	**1.76**	**1.76**	**1.79**	**1.79**	**1.67**	**1.71**	**1.77**	**1.77**
SD	0.27	0.23	0.22	0.20	0.24	0.25	0.21	0.27	0.18	0.34	0.18	0.21
RSD	15.3	12.3	11.3	10.9	13.5	14.4	11.5	14.8	11.0	20.1	10.1	12.1
Total mean				**1.85**				**1.78**				**1.73(*)**
Total SD				0.05				0.02				0.05
Total RSD				2.6				1.0				2.8

(*) Statistically significant reduced compared to control, $p<0.05$, Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm, one-sided smaller, heterogeneous variances.

Table 45: F_1 generation, early life stage: individual total length at day 35 pf [cm]; 32.0, 100 and 320 ng Dienogest/L (nominal), respective 31.7, 105 and 335 ng Dienogest/L (mean measured)

	Nominal concentration Dienogest [ng/L]											
	32.0				100				320			
	Mean measured concentration Dienogest [ng/L]											
	31.7				105				335			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Individual total length day 35 pf [cm]											
1	1.86	1.76	1.83	1.76	-	1.68	2.11	1.50	2.10	2.21	1.29	1.87
2	1.82	2.03	1.84	2.14	-	2.01	2.03	1.98	2.19	2.26	1.80	1.99
3	1.84	1.89	1.80	1.99	-	2.03	2.21	1.86	1.88	2.16	1.95	1.62
4	1.55	1.92	1.45	1.74	-	1.88	1.94	1.67	1.64	1.84	1.65	1.90
5	1.94	1.92	2.09	1.35	-	1.38	2.23	2.02	2.07	1.74	1.96	1.62
6	1.89	1.70	2.00	2.11	-	2.23	2.17	1.93	2.08	2.29	1.82	1.66
7	1.90	1.65	1.41	1.56	-	1.78	2.10	1.68	2.03	2.12	1.78	1.96
8	1.92	1.70	1.85	2.03	-	1.85	-	1.67	2.25	2.27	1.85	1.96
9	1.91	1.74	1.74	1.44	-	2.16	-	1.95	2.40	2.11	1.44	1.85
10	1.54	2.18	1.71	2.06	-	1.77	-	1.94	2.22	-	1.85	1.59
11	1.94	1.96	1.43	1.92	-	1.98	-	1.50	2.12	-	1.81	2.00
12	2.04	1.82	2.02	1.88	-	1.36	-	1.38	1.91	-	1.92	2.22
13	1.65	2.07	1.86	1.70	-	1.31	-	1.89	-	-	2.02	1.88
14	1.88	1.90	1.82	2.08	-	1.90	-	1.77	-	-	1.93	1.94
15	1.85	1.75	1.58	1.57	-	1.53	-	1.89	-	-	2.04	1.35
16	1.61	2.11	2.03	2.11	-	1.76	-	1.69	-	-	1.76	1.62
17	1.53	1.58	1.90	2.08	-	1.80	-	2.33	-	-	1.91	1.99
18	1.68	1.85	1.83	1.74	-	1.65	-	-	-	-	1.48	1.82
19	1.58	1.38	1.86	2.01	-	1.24	-	-	-	-	2.13	-
20	1.70	1.55	-	2.02	-	1.49	-	-	-	-	1.53	-
21	1.40	2.00	-	1.63	-	1.75	-	-	-	-	1.43	-
22	1.68	1.53	-	1.55	-	1.46	-	-	-	-	1.20	-
23	1.97	1.57	-	1.81	-	1.49	-	-	-	-	1.79	-
24	1.92	1.34	-	1.70	-	1.98	-	-	-	-	2.12	-
25	-	1.56	-	1.48	-	1.72	-	-	-	-	1.71	-

	Nominal concentration Dienogest [ng/L]											
26	-	1.95	-	1.38	-	1.21	-	-	-	-	1.95	-
27	-	1.60	-	1.64	-	1.86	-	-	-	-	1.86	-
28	-	2.14	-	1.28	-	1.81	-	-	-	-	2.00	-
29	-	1.95	-	1.71	-	2.03	-	-	-	-	1.58	-
30	-	1.78	-	-	-	1.82	-	-	-	-	-	-
31	-	1.64	-	-	-	-	-	-	-	-	-	-
Mean	**1.78**	**1.79**	**1.79**	**1.77**	**-**	**1.73**	**2.11**	**1.80**	**2.07**	**2.11**	**1.78**	**1.82**
SD	0.17	0.22	0.20	0.26	-	0.27	0.10	0.23	0.20	0.19	0.24	0.21
RSD	9.8	12.2	11.2	14.5	-	15.6	4.9	12.8	9.5	9.2	13.5	11.4
Total mean				**1.78**				**1.88**				**1.95**
Total SD				0.01				0.20				0.17
Total RSD				0.5				10.8				8.7

C.1.7 First filial generation (F_1), juvenile growth

Table 46: F_1 generation, juvenile growth: survival at day 63 pf

		Nominal concentration Dienogest [ng/L]					
		Control	3.20	10.0	32.0	100	320
		Mean measured concentration Dienogest [ng/L]					
	Replicate	Control	3.51	10.3	31.7	105	335
Fish numbers after reduction at day 35 pf, [n]	A	20	20	20	20	-	12
	B	20	20	20	20	20	9
	C	20	20	20	19	7	20
	D	20	20	20	20	17	18
Survived, day 63 pf [n]	A	20	20	20	20	-	12
	B	20	20	20	20	20	9
	C	19	20	20	19	7	20
	D	20	20	20	20	17	18
Survived, day 63 pf [%]	A	100	100	100	100	-	100
	B	100	100	100	100	100	100
	C	95.0	100	100	100	100	100
	D	100	100	100	100	100	100
	Mean	**98.8**	**100**	**100**	**100**	**100**	**100**
	SD	2.5	0.0	0.0	0.0	0.0	0.0
	RSD	2.5	0.0	0.0	0.0	0.0	0.0

Table 47: F_1 generation, juvenile growth: individual total length at day 63 pf [cm]; control, 3.20 and 10.0 ng Dienogest/L (nominal), respective 3.51 and 10.3 ng Dienogest/L (mean measured)

	Nominal concentration Dienogest [ng/L]											
	Control				3.20				10.0			
	Mean measured concentration Dienogest [ng/L]											
	Control				3.51				10.3			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Individual total length day 63 pf [cm]**											
1	2.93	3.22	3.02	3.03	3.02	3.35	3.05	2.95	2.94	2.52	3.14	2.87
2	2.95	3.38	3.63	3.17	3.12	2.90	3.47	3.13	2.72	3.27	3.07	3.31
3	3.08	3.08	3.17	2.95	2.89	2.83	3.08	3.24	3.05	2.89	3.38	3.26
4	3.10	2.99	3.03	3.12	3.45	3.09	2.97	2.40	3.30	2.73	3.06	3.15
5	3.34	3.33	3.14	3.25	2.64	3.39	3.04	3.00	3.08	3.03	3.26	3.03
6	3.06	3.23	3.32	2.95	2.97	3.28	2.90	3.34	3.24	3.12	3.03	3.02
7	2.98	3.01	2.76	2.94	3.35	3.09	3.28	3.02	3.02	3.11	2.97	3.03
8	2.94	3.33	3.13	2.83	3.29	3.33	3.05	3.05	3.17	3.19	3.16	3.07
9	2.91	2.94	3.20	3.16	3.19	3.04	3.30	3.16	3.09	2.81	2.80	2.87
10	2.87	3.10	3.08	3.34	2.86	2.98	2.54	3.01	3.19	3.27	3.29	3.08
11	2.88	3.12	3.34	3.03	2.95	3.15	2.85	3.15	3.12	3.19	2.74	2.96
12	2.90	2.96	3.23	3.35	3.04	3.39	2.94	3.24	3.14	3.13	3.28	3.03
13	2.80	3.36	3.43	3.08	3.15	3.47	3.51	3.29	3.06	3.19	3.02	3.24
14	3.21	2.93	3.16	3.07	2.94	2.94	3.41	2.68	3.04	2.98	3.40	2.85
15	2.80	3.11	3.23	3.12	3.26	3.06	3.04	3.35	3.55	2.64	3.17	3.04
16	3.13	3.07	3.22	3.33	3.27	3.06	3.08	3.48	3.07	3.29	3.10	3.45
17	2.92	3.32	2.90	2.86	3.28	3.31	3.08	3.32	3.17	2.94	2.85	3.43
18	3.25	3.10	3.14	2.94	3.06	2.90	3.38	2.99	3.05	3.14	3.17	3.24
19	3.15	2.84	-	3.10	3.03	3.05	3.19	3.14	3.03	3.11	3.18	3.21
20	3.15	3.22	-	3.39	3.03	2.90	3.30	3.11	3.17	3.46	3.17	3.46
21	-	-	-	3.27(*)	-	-	-	-	-	-	-	-
Mean	**3.02**	**3.13**	**3.17**	**3.11**	**3.09**	**3.13**	**3.12**	**3.10**	**3.11**	**3.05**	**3.11**	**3.13**
SD	0.15	0.16	0.19	0.17	0.19	0.20	0.24	0.24	0.16	0.24	0.18	0.19
RSD	5.1	5.1	6.1	5.4	6.3	6.3	7.6	7.8	5.1	7.7	5.7	6.0
Total mean	**3.11**				**3.11**				**3.10**			
Total SD	0.07				0.02				0.03			
Total RSD	2.1				0.6				1.1			

(*) Surplus fish of the same treatment (=control), most probably due to a handling mistake during photographing (unintential transfer from replicate C to D).

Table 48: F_1 generation, early life stage: individual total length at day 63 pf [cm]; 32.0, 100 and 320 ng Dienogest/L (nominal), respective 31.7, 105 and 335 ng Dienogest/L (mean measured)

	Nominal concentration Dienogest [ng/L]											
	32.0				100				320			
	Mean measured concentration Dienogest [ng/L]											
	31.7				105				335			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Individual total length day 63 pf [cm]											
1	2.94	2.78	3.29	3.26	-	2.92	3.59	2.94	3.46	3.24	2.97	3.07
2	2.96	3.09	2.92	3.32	-	3.22	3.42	3.25	3.32	3.57	3.09	3.20
3	3.32	3.09	3.32	3.47	-	3.35	3.71	3.50	3.51	3.69	3.05	2.86
4	2.92	3.11	3.23	3.14	-	3.20	3.71	3.21	3.38	3.29	2.95	3.10
5	3.46	2.77	3.17	3.27	-	3.24	3.50	3.21	3.38	3.63	3.06	3.00
6	3.45	3.26	3.28	3.27	-	3.16	3.75	3.09	3.47	3.53	3.06	2.95
7	2.99	3.19	3.18	3.12	-	2.81	3.77	3.11	3.01	3.61	3.20	3.19
8	3.18	3.08	3.09	2.69	-	2.96	-	2.86	3.10	3.49	3.04	2.94
9	3.08	3.21	2.91	2.80	-	3.03	-	3.03	3.49	3.11	3.09	3.13
10	3.04	3.14	3.16	2.66	-	3.35	-	3.01	3.35	-	2.97	3.26
11	3.06	3.08	2.96	2.92	-	2.76	-	3.08	3.33	-	2.95	3.07
12	3.26	3.24	3.18	2.78	-	3.01	-	3.27	3.16	-	2.57	3.46
13	2.97	3.16	3.34	3.13	-	1.64	-	3.36	-	-	2.89	2.93
14	3.14	2.84	3.05	3.27	-	3.14	-	3.27	-	-	2.96	2.86
15	2.90	3.33	2.80	3.29	-	3.10	-	2.89	-	-	3.07	3.08
16	3.18	2.94	2.90	2.94	-	2.95	-	3.43	-	-	3.32	2.76
17	3.10	3.11	3.31	3.28	-	3.19	-	3.31	-	-	3.19	3.32
18	3.24	3.45	2.90	3.34	-	2.42	-	-	-	-	2.82	3.20
19	2.84	2.98	3.12	3.13	-	2.92	-	-	-	-	3.02	-
20	2.87	2.89	-	3.28	-	3.16	-	-	-	-	2.85	-
Mean	**3.10**	**3.09**	**3.11**	**3.12**	**-**	**2.98**	**3.64**	**3.17**	**3.33**	**3.46**	**3.01**	**3.08**
SD	0.18	0.18	0.17	0.24	-	0.38	0.13	0.19	0.16	0.20	0.16	0.18
RSD	5.9	5.8	5.4	7.6	-	12.9	3.7	5.9	4.8	5.8	5.2	5.8
Total mean				**3.10**				**3.26**				**3.22**
Total SD				0.01				0.34				0.21
Total RSD				0.5				10.4				6.6

C.1.8 First filial generation (F_1), reproduction

Table 49: F_1 generation, reproduction: fecundity and fertilisation rate; control

	Control																			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Time of exposure [day]	Fertilised eggs [n]				Total egg number [n]				Fertilisation rate [%]				Female number [n]				Total egg number per female [n]			
64	0	303	437	302	0	327	518	321		92.7	84.4	94.1	7	11	6	7	0	30	86	46
65	108	118	86	32	122	124	92	35	88.5	95.2	93.5	91.4	7	11	6	7	17	11	15	5
66	101	205	318	120	120	312	337	133	84.2	65.7	94.4	90.2	7	11	6	7	17	28	56	19
67	240	326	29	65	274	384	31	70	87.6	84.9	93.5	92.9	7	11	6	7	39	35	5	10
68	114	69	528	240	123	74	575	261	92.7	93.2	91.8	92.0	7	11	6	7	18	7	96	37
69	100	273	128	179	102	348	130	189	98.0	78.4	98.5	94.7	7	11	6	7	15	32	22	27
70	260	227	185	103	271	236	198	113	95.9	96.2	93.4	91.2	7	11	6	7	39	21	33	16
71	101	242	151	220	104	265	154	272	97.1	91.3	98.1	80.9	7	11	6	7	15	24	26	39
72	505	161	188	99	626	306	225	147	80.7	52.6	83.6	67.3	7	11	6	7	89	28	38	21
73	0	177	306	105	0	269	367	123	-	65.8	83.4	85.4	7	11	6	7	0	24	61	18
74	383	114	90	222	392	161	112	249	97.7	70.8	80.4	89.2	7	11	6	7	56	15	19	36
75	138	124	191	138	158	219	225	169	87.3	56.6	84.9	81.7	7	11	6	7	23	20	38	24
76	641	290	44	123	749	309	78	149	85.6	93.9	56.4	82.6	7	11	6	7	107	28	13	21
77	0	69	153	75	0	78	184	83	-	88.5	83.2	90.4	7	11	6	7	0	7	31	12

	Control																			
78	307	416	268	180	346	567	314	204	88.7	73.4	85.4	88.2	7	11	6	7	49	52	52	29
79	157	110	310	116	176	117	357	124	89.2	94.0	86.8	93.5	7	11	6	7	25	11	60	18
80	388	388	0	60	401	457	0	70	96.8	84.9	-	85.7	7	11	6	7	57	42	0	10
81	102	35	227	237	126	43	262	291	81.0	81.4	86.6	81.4	7	11	6	7	18	4	44	42
82	556	249	240	142	657	299	281	166	84.6	83.3	85.4	85.5	7	11	6	7	94	27	47	24
83	293	35	254	236	333	39	299	242	88.0	89.7	84.9	97.5	7	11	6	7	48	4	50	35
84	351	417	216	354	379	480	237	369	92.6	86.9	91.1	95.9	7	11	6	7	54	44	40	53
85	91	50	471	276	111	54	566	323	82.0	92.6	83.2	85.4	7	11	6	7	16	5	94	46
86	193	121	10	175	237	141	14	190	81.4	85.8	71.4	92.1	7	11	6	7	34	13	2	27
Mean	**-**	**-**	**-**	**-**	**273**	**254**	**237**	**171**	**89.8**	**81.3**	**86.8**	**87.8**	**-**	**-**	**-**	**-**	**39**	**23**	**39**	**24**
SD	-	-	-	-	214	150	150	81	5.6	13.1	9.1	6.8	-	-	-	-	31	14	25	12
RSD	-	-	-	-	78.4	59.0	63.5	47.5	6.3	16.1	10.5	7.8	-	-	-	-	78.4	59.0	63.5	47.5
Cumulative egg number [n]					**5807**	**5609**	**5556**	**4293**												

Green marked table fields: Counting period of 20 days, starting with at least 15 eggs and fertility ≥80% in the controls on three consecutive days.

Mean values of egg number, fertilization rate and total egg number per female consider the numbers of the green marked fields, only.

Table 50: F_1 generation, reproduction: fecundity and fertilisation rate; 3.20 ng Dienogest/L (nominal), respective 3.51 ng Dienogest/L (mean measured)

	Nominal concentration: 3.20 ng Dienogest/L Mean measured concentration: 3.51 ng Dienogest/L																			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Time of exposure [day]	**Fertilised eggs [n]**				**Total egg number [n]**				**Fertilisation rate [%]**				**Female number [n]**				**Total egg number per female [n]**			
64	220	398	248	0	244	439	266	0	90.2	90.7	93.2	-	9	10	6	9	27	44	44	0
65	288	267	150	187	324	295	160	215	88.9	90.5	93.8	87.0	9	10	6	9	36	30	27	24
66	197	240	33	31	210	251	37	33	93.8	95.6	89.2	93.9	9	10	6	9	23	25	6	4
67	366	185	141	393	401	203	152	432	91.3	91.1	92.8	91.0	9	10	6	9	45	20	25	48
68	74	238	179	102	85	248	238	131	87.1	96.0	75.2	77.9	9	10	6	9	9	25	40	15
69	193	358	188	227	219	373	216	255	88.1	96.0	87.0	89.0	9	10	6	9	24	37	36	28
70	188	478	120	168	218	514	129	185	86.2	93.0	93.0	90.8	9	10	6	9	24	51	22	21
71	363	344	110	193	374	350	120	207	97.1	98.3	91.7	93.2	9	10	6	9	42	35	20	23
72	129	380	110	191	156	442	132	270	82.7	86.0	83.3	70.7	9	10	6	9	17	44	22	30
73	359	426	148	259	388	488	161	299	92.5	87.3	91.9	86.6	9	10	6	9	43	49	27	33
74	273	523	105	107	293	547	120	116	93.2	95.6	87.5	92.2	9	10	6	9	33	55	20	13
75	12	351	138	312	13	419	157	373	92.3	83.8	87.9	83.6	9	10	6	9	1	42	26	41
76	489	355	150	98	596	379	180	121	82.0	93.7	83.3	81.0	9	10	6	9	66	38	30	13
77	360	185	45	251	388	197	47	301	92.8	93.9	95.7	83.4	9	10	6	9	43	20	8	33
78	287	504	180	196	313	530	194	206	91.7	95.1	92.8	95.1	9	10	6	9	35	53	32	23

	Nominal concentration: 3.20 ng Dienogest/L **Mean measured concentration: 3.51 ng Dienogest/L**																			
79	374	346	97	54	393	368	104	64	95.2	94.0	93.3	84.4	9	10	6	9	44	37	17	7
80	325	101	86	274	352	128	101	464	92.3	78.9	85.1	59.1	9	10	6	9	39	13	17	52
81	143	978	108	167	178	1062	127	200	80.3	92.1	85.0	83.5	9	10	6	9	20	106	21	22
82	281	491	123	91	300	511	256	105	93.7	96.1	48.0	86.7	9	10	6	9	33	51	43	12
83	152	199	26	158	159	201	29	170	95.6	99.0	89.7	92.9	9	10	6	9	18	20	5	19
84	592	350	35	128	619	365	45	135	95.6	95.9	77.8	94.8	9	10	6	9	69	37	8	15
85	462	456	392	107	484	472	451	112	95.5	96.6	86.9	95.5	9	10	6	9	54	47	75	12
86	259	614	137	180	284	656	203	205	91.2	93.6	67.5	87.8	9	10	6	9	32	66	34	23
Mean	**-**	**-**	**-**	**-**	**299**	**394**	**135**	**214**	**90.6**	**92.6**	**86.2**	**85.8**	**-**	**-**	**-**	**-**	**33**	**39**	**23**	**24**
SD	-	-	-	-	152	201	64	116	4.8	5.1	10.5	8.9	-	-	-	-	17	20	11	13
RSD	-	-	-	-	50.8	51.1	47.6	54.3	5.3	5.5	12.2	10.3	-	-	-	-	50.8	51.1	47.6	54.3
Cumulative egg number [n]					**6991**	**9438**	**3625**	**4599**												

Green marked table fields: Counting period of 20 days, starting with at least 15 eggs and fertility ≥80% in the controls on three consecutive days.

Mean values of egg number, fertilization rate and total egg number per female consider the numbers of the green marked fields, only.

Table 51: F_1 generation, reproduction: fecundity and fertilisation rate; 10.0 ng Dienogest/L (nominal), respective 10.3 ng Dienogest/L (mean measured)

	Nominal concentration: 10.0 ng Dienogest/L Mean measured concentration: 10.3 ng Dienogest/L																			
Replikate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Time of exposure [day]	**Fertilised eggs [n]**				**Total egg number [n]**				**Fertilisation rate [%]**				**Female number [n]**				**Total egg number per female [n]**			
64	105	457	122	78	112	509	171	90	93.8	89.8	71.3	86.7	14	11	8	12	8	46	21	8
65	122	95	39	119	139	100	40	130	87.8	95.0	97.5	91.5	14	11	8	12	10	9	5	11
66	195	309	226	0	214	326	234	0	91.1	94.8	96.6	-	14	11	8	12	15	30	29	0
67	202	171	18	123	226	189	22	145	89.4	90.5	81.8	84.8	14	11	8	12	16	17	3	12
68	117	349	416	64	124	391	492	68	94.4	89.3	84.6	94.1	14	11	8	12	9	36	62	6
69	291	140	164	79	320	147	176	89	90.9	95.2	93.2	88.8	14	11	8	12	23	13	22	7
70	190	384	256	140	208	430	280	152	91.3	89.3	91.4	92.1	14	11	8	12	15	39	35	13
71	126	284	219	151	137	315	223	169	92.0	90.2	98.2	89.3	14	11	8	12	10	29	28	14
72	345	359	198	231	434	381	223	328	79.5	94.2	88.8	70.4	14	11	8	12	31	35	28	27
73	297	101	187	161	340	103	206	219	87.4	98.1	90.8	73.5	14	11	8	12	24	9	26	18
74	458	664	309	230	508	710	335	299	90.2	93.5	92.2	76.9	14	11	8	12	36	65	42	25
75	277	131	234	282	315	151	254	339	87.9	86.8	92.1	83.2	14	11	8	12	23	14	32	28
76	353	186	412	148	374	199	444	178	94.4	93.5	92.8	83.1	14	11	8	12	27	18	56	15
77	300	376	100	353	352	407	118	421	85.2	92.4	84.7	83.8	14	11	8	12	25	37	15	35
78	250	622	295	336	280	691	323	391	89.3	90.0	91.3	85.9	14	11	8	12	20	63	40	33

	Nominal concentration: 10.0 ng Dienogest/L **Mean measured concentration: 10.3 ng Dienogest/L**																			
79	137	68	65	199	151	74	70	226	90.7	91.9	92.9	88.1	14	11	8	12	11	7	9	19
80	334	347	547	468	431	465	611	523	77.5	74.6	89.5	89.5	14	11	8	12	31	42	76	44
81	156	322	124	93	175	412	142	121	89.1	78.2	87.3	76.9	14	11	8	12	13	37	18	10
82	174	420	586	467	200	482	682	577	87.0	87.1	85.9	80.9	14	11	8	12	14	44	85	48
83	237	171	106	304	290	212	124	343	81.7	80.7	85.5	88.6	14	11	8	12	21	19	16	29
84	330	439	441	125	342	461	476	135	96.5	95.2	92.6	92.6	14	11	8	12	24	42	60	11
85	538	427	219	338	577	450	231	375	93.2	94.9	94.8	90.1	14	11	8	12	41	41	29	31
86	239	402	251	143	271	426	288	161	88.2	94.4	87.2	88.8	14	11	8	12	19	39	36	13
Mean	**-**	**-**	**-**	**-**	**278**	**332**	**274**	**243**	**88.7**	**90.0**	**90.5**	**85.0**	**-**	**-**	**-**	**-**	**20**	**30**	**34**	**20**
SD	-	-	-	-	111	185	185	155	4.8	6.1	4.5	6.7	-	-	-	-	8	17	23	13
RSD	-	-	-	-	39.9	55.6	67.5	63.7	5.4	6.8	4.9	7.9	-	-	-	-	39.9	55.6	67.5	63.7
Cumulative egg number [n]					**6520**	**8031**	**6165**	**5479**												

Green marked table fields: Counting period of 20 days, starting with at least 15 eggs and fertility ≥80% in the controls on three consecutive days.

Mean values of egg number, fertilization rate and total egg number per female consider the numbers of the green marked fields, only.

Table 52: F_1 generation, reproduction: fecundity and fertilisation rate; 32.0 ng Dienogest/L (nominal), respective 31.7 ng Dienogest/L (mean measured)

	Nominal concentration: 32.0 ng Dienogest/L Mean measured concentration: 31.7 ng Dienogest/L																			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Time of exposure [day]	Fertilised eggs [n]				Total egg number [n]				Fertilisation rate [%]				Female number [n]				Total egg number per female [n]			
64	176	65	213	110	190	70	219	112	92.6	92.9	97.3	98.2	8	5	12	12	24	14	18	9
65	58	45	141	305	69	59	156	349	84.1	76.3	90.4	87.4	8	5	12	12	9	12	13	29
66	221	0	324	331	266	0	370	408	83.1	-	87.6	81.1	8	5	12	12	33	0	31	34
67	240	137	321	291	262	151	345	352	91.6	90.7	93.0	82.7	8	5	12	12	33	30	29	29
68	125	20	530	326	147	24	602	427	85.0	83.3	88.0	76.3	8	5	12	12	18	5	50	36
69	265	242	666	386	285	271	802	470	93.0	89.3	83.0	82.1	8	5	12	12	36	54	67	39
70	231	5	396	575	260	6	455	622	88.8	83.3	87.0	92.4	8	5	12	12	33	1	38	52
71	284	194	595	384	306	249	677	403	92.8	77.9	87.9	95.3	8	5	12	12	38	50	56	34
72	185	66	543	561	207	79	923	673	89.4	83.5	58.8	83.4	8	5	12	12	26	16	77	56
73	91	0	352	288	115	0	469	410	79.1	-	75.1	70.2	8	5	12	12	14	0	39	34
74	525	240	809	552	558	253	958	625	94.1	94.9	84.4	88.3	8	5	12	12	70	51	80	52
75	247	4	302	359	288	6	436	492	85.8	66.7	69.3	73.0	8	5	12	12	36	1	36	41
76	138	117	761	364	146	143	1002	387	94.5	81.8	75.9	94.1	8	5	12	12	18	29	84	32
77	208	43	124	383	337	111	171	441	61.7	38.7	72.5	86.8	8	5	12	12	42	22	14	37
78	320	142	809	618	338	146	938	792	94.7	97.3	86.2	78.0	8	5	12	12	42	29	78	66

	Nominal concentration: 32.0 ng Dienogest/L Mean measured concentration: 31.7 ng Dienogest/L																			
79	181	175	395	584	210	196	482	638	86.2	89.3	82.0	91.5	8	5	12	12	26	39	40	53
80	354	31	747	728	380	34	904	917	93.2	91.2	82.6	79.4	8	5	12	12	48	7	75	76
81	52	224	362	232	63	250	471	296	82.5	89.6	76.9	78.4	8	5	12	12	8	50	39	25
82	446	59	464	570	513	74	629	694	86.9	79.7	73.8	82.1	8	5	12	12	64	15	52	58
83	188	184	333	297	208	204	363	320	90.4	90.2	91.7	92.8	8	5	12	12	26	41	30	27
84	153	185	474	472	178	200	506	544	86.0	92.5	93.7	86.8	8	5	12	12	22	40	42	45
85	354	109	424	718	402	138	493	795	88.1	79.0	86.0	90.3	8	5	12	12	50	28	41	66
86	223	29	582	416	240	31	640	468	92.9	93.5	90.9	88.9	8	5	12	12	30	6	53	39
Mean	**-**	**-**	**-**	**-**	**257**	**123**	**583**	**513**	**87.1**	**83.1**	**82.0**	**84.1**	**-**	**-**	**-**	**-**	**32**	**25**	**49**	**43**
SD	-	-	-	-	130	96	262	169	7.5	13.4	9.0	7.1	-	-	-	-	16	19	22	14
RSD	-	-	-	-	50.5	78.0	44.9	32.9	8.6	16.1	11.0	8.4	-	-	-	-	50.5	78.0	44.9	32.9
Cumulative egg number [n]					**5968**	**2695**	**13011**	**11635**												

Green marked table fields: counting period of 20 days, starting with at least 15 eggs and fertility ≥80% in the controls on three consecutive days

Mean values of egg number, fertilization rate and total egg number per female consider the numbers of the green marked fields, only.

Table 53: F_1 generation, reproduction: fecundity and fertilisation rate; 100 ng Dienogest/L (nominal), respective 105 ng Dienogest/L (mean measured)

	Nominal concentration: Mean measured concentration:	100 ng Dienogest/L 105 ng Dienogest/L																		
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Time of exposure [day]	**Fertilised eggs [n]**				**Total egg number [n]**				**Fertilisation rate [%]**				**Female number [n]**				**Total egg number per female [n]**			
64	-	439	48	320	-	445	56	332	-	98.7	85.7	96.4	-	11	3	12	-	40	19	28
65	-	98	8	183	-	99	24	209	-	99.0	33.3	87.6	-	11	3	12	-	9	8	17
66	-	0	96	77	-	0	181	152	-		53.0	50.7	-	11	3	12	-	0	60	13
67	-	244	200	575	-	281	241	668	-	86.8	83.0	86.1	-	11	3	12	-	26	80	56
68	-	109	112	576	-	118	123	670	-	92.4	91.1	86.0	-	11	3	12	-	11	41	56
69	-	362	154	362	-	391	170	383	-	92.6	90.6	94.5	-	11	3	12	-	36	57	32
70	-	239	152	478	-	270	164	540	-	88.5	92.7	88.5	-	11	3	12	-	25	55	45
71	-	354	247	553	-	368	268	659	-	96.2	92.2	83.9	-	11	3	12	-	33	89	55
72	-	77	95	325	-	86	216	440	-	89.5	44.0	73.9	-	11	3	12	-	8	72	37
73	-	251	140	517	-	263	161	631	-	95.4	87.0	81.9	-	11	3	12	-	24	54	53
74	-	533	97	712	-	566	211	815	-	94.2	46.0	87.4	-	11	3	12	-	51	70	68
75	-	462	279	449	-	489	324	586	-	94.5	86.1	76.6	-	11	3	12	-	44	108	49
76	-	238	29	718	-	246	45	886	-	96.7	64.4	81.0	-	11	3	12	-	22	15	74
77	-	363	177	150	-	412	429	256	-	88.1	41.3	58.6	-	11	3	12	-	37	143	21
78	-	509	87	908	-	544	171	1022	-	93.6	50.9	88.8	-	11	3	12	-	49	57	85

	Nominal concentration: **Mean measured concentration:**				**100 ng Dienogest/L** **105 ng Dienogest/L**															
79	-	200	59	461	-	223	137	512	-	89.7	43.1	90.0	-	11	3	12	-	20	46	43
80	-	908	260	598	-	987	347	662	-	92.0	74.9	90.3	-	11	3	12	-	90	116	55
81	-	262	177	580	-	279	274	678	-	93.9	64.6	85.5	-	11	3	12	-	25	91	57
82	-	426	67	661	-	480	109	821	-	88.8	61.5	80.5	-	11	3	12	-	44	36	68
83	-	618	199	526	-	648	247	577	-	95.4	80.6	91.2	-	11	3	12	-	59	82	48
84	-	250	113	434	-	275	246	471	-	90.9	45.9	92.1	-	11	3	12	-	25	82	39
85	-	460	77	368	-	495	128	395	-	92.9	60.2	93.2	-	11	3	12	-	45	43	33
86	-	244	474	19	-	252	522	69	-	96.8	90.8	27.5	-	11	3	12	-	23	174	6
Mean	**-**	**-**	**-**	**-**	**-**	**351**	**204**	**582**	**-**	**92.5**	**66.3**	**82.8**	**-**	**-**	**-**	**-**		**32**	**68**	**48**
SD	-	-	-	-	-	228	98	223	-	3.3	20.5	11.0	-	-	-	-		21	33	19
RSD	-	-	-	-	-	64.9	48.1	38.3	-	3.6	30.9	13.3	-	-	-	-		64.9	48.1	38.3
Cumulative egg number [n]					**-**	**8217**	**4794**	**12434**												

Green marked table fields: Counting period of 20 days, starting with at least 15 eggs and fertility ≥80% in the controls on three consecutive days.

Mean values of egg number, fertilization rate and total egg number per female consider the numbers of the green marked fields, only.

Table 54: F_1 generation, reproduction: fecundity and fertilisation rate; 320 ng Dienogest/L (nominal), respective 335 ng Dienogest/L (mean measured)

	Nominal concentration: 320 ng Dienogest/L Mean measured concentration: 335 ng Dienogest/L																			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Time of exposure [day]	**Fertilised eggs [n]**				**Total egg number [n]**				**Fertilisation rate [%]**				**Female number [n]**				**Total egg number per female [n]**			
64	76	291	209	100	86	313	281	107	88.4	93.0	74.4	93.5	4	6	9	7	22	52	31	15
65	183	361	140	97	209	399	178	110	87.6	90.5	78.7	88.2	4	6	9	7	52	67	20	16
66	66	188	50	160	85	198	70	173	77.6	94.9	71.4	92.5	4	6	9	7	21	33	8	25
67	260	371	157	102	283	447	220	121	91.9	83.0	71.4	84.3	4	6	9	7	71	75	24	17
68	71	369	188	281	139	406	207	306	51.1	90.9	90.8	91.8	4	6	9	7	35	68	23	44
69	244	381	231	118	305	478	293	128	80.0	79.7	78.8	92.2	4	6	9	7	76	80	33	18
70	193	163	155	209	239	174	167	219	80.8	93.7	92.8	95.4	4	6	9	7	60	29	19	31
71	118	246	256	254	193	294	316	342	61.1	83.7	81.0	74.3	4	6	9	7	48	49	35	49
72	109	118	108	163	212	310	194	338	51.4	38.1	55.7	48.2	4	6	9	7	53	52	22	48
73	90	411	144	151	161	537	196	198	55.9	76.5	73.5	76.3	4	6	9	7	40	90	22	28
74	124	448	273	274	246	533	314	329	50.4	84.1	86.9	83.3	4	6	9	7	62	89	35	47
75	44	246	68	320	148	268	94	363	29.7	91.8	72.3	88.2	4	6	9	7	37	45	10	52
76	4	149	224	271	116	688	356	305	3.4	21.7	62.9	88.9	4	6	9	7	29	115	40	44
77	116	60	92	36	236	368	111	81	49.2	16.3	82.9	44.4	4	6	9	7	59	61	12	12
78	20	225	220	531	147	389	239	603	13.6	57.8	92.1	88.1	4	6	9	7	37	65	27	86

	Nominal concentration: 320 ng Dienogest/L **Mean measured concentration: 335 ng Dienogest/L**																			
79	123	382	358	76	317	424	448	99	38.8	90.1	79.9	76.8	4	6	9	7	79	71	50	14
80	15	83	332	436	82	209	401	532	18.3	39.7	82.8	82.0	4	6	9	7	21	35	45	76
81	130	149	235	132	235	258	320	143	55.3	57.8	73.4	92.3	4	6	9	7	59	43	36	20
82	138	160	210	219	201	372	251	275	68.7	43.0	83.7	79.6	4	6	9	7	50	62	28	39
83	62	250	163	293	67	313	186	308	92.5	79.9	87.6	95.1	4	6	9	7	17	52	21	44
84	196	271	145	230	301	294	168	253	65.1	92.2	86.3	90.9	4	6	9	7	75	49	19	36
85	156	211	393	302	201	255	558	406	77.6	82.7	70.4	74.4	4	6	9	7	50	43	62	58
86	112	231	144	139	140	252	211	206	80.0	91.7	68.2	67.5	4	6	9	7	35	42	23	29
Mean	**-**	**-**	**-**	**-**	**196**	**368**	**236**	**261**	**56.1**	**70.3**	**79.2**	**82.6**	**-**	**-**	**-**	**-**	**49**	**61**	**26**	**37**
SD	-	-	-	-	76	129	101	140	25.8	25.5	9.7	13.9	-	-	-	-	19	21	11	20
RSD	-	-	-	-	38.9	34.9	42.6	53.4	45.9	36.3	12.2	16.9	-	-	-	-	38.9	34.9	42.6	53.4
Cumulative egg number [n]					**4349**	**8179**	**5779**	**5945**												

Green marked table fields: Counting period of 20 days, starting with at least 15 eggs and fertility ≥80% in the controls on three consecutive days.

Mean values of egg number, fertilization rate and total egg number per female consider the numbers of the green marked fields, only.

Table 55: F_1 generation, summary: reproduction

		Nominal concentration Dienogest [ng/L]					
		Control	**3.20**	**10.0**	**32.0**	**100**	**320**
		Mean measured concentration Dienogest [ng/L]					
	Replicate	**Control**	**3.51**	**10.3**	**31.7**	**105**	**335**
Total eggs per day and female [n]	A	39	33	20	32	-	49
	B	23	39	30	25	32	61
	C	39	23	34	49	68	26
	D	24	24	20	43	48	37
	Mean	**31**	**30**	**26**	**37**	**50**	**43**
	SD	9	8	7	11	18	15
	RSD	28.5	26.9	27.6	29.0	36.6	34.7
Fertilisation rate [%]	A	89.8	90.6	88.7	87.1	-	56.1
	B	81.3	92.6	90.0	83.1	92.5	70.3
	C	86.8	86.2	90.5	82.0	66.3	79.2
	D	87.8	85.8	85.0	84.1	82.8	82.6
	Mean	**86.4**	**88.8**	**88.5**	**84.1**	**80.5**	**72.1(*)**
	SD	3.6	3.3	2.5	2.2	13.3	11.8
	RSD	4.2	3.7	2.8	2.6	16.5	16.4

(*) Statistically significant reduction compared to control, p<0.05, Williams test, one-sided smaller.

C.1.9 First filial generation (F_1), adult stage

Table 56: F_1 generation, adult stage: survival

		Nominal concentration Dienogest [ng/L]					
		Control	**3.20**	**10.0**	**32.0**	**100**	**320**
		Mean measured concentration Dienogest [ng/L]					
	Replicate	**Control**	**3.51**	**10.3**	**31.7**	**105**	**335**
Fish number, day 63 pf [n]	A	20	20	20	20	-	12
	B	20	20	20	20	20	9
	C	19	20	20	19	7	20
	D	20	20	20	20	17	18
Survival, adult stage [n]	A	20	20	20	20	-	11
	B	20	19	20	16	19	9
	C	19	20	20	19	7	20
	D	20	20	20	20	17	18
Survival, adult stage [%]	A	100	100	100	100.0	-	91.7
	B	100	95.0	100	80.0	95.0	100
	C	100	100	100	100	100	100
	D	100	100	100	100	100	100
	Mean	**100**	**98.8**	**100**	**95.0**	**98.3**	**97.9**
	SD	0.0	2.5	0.0	10.0	2.9	4.2
	RSD	0.0	2.5	0.0	10.5	2.9	4.3

Table 57: F_1 generation, adult stage: individual total length [cm]; control and 3.20 ng Dienogest/L, respective 3.51 ng Dienogest/L (mean measured)

	Control								Nominal concentration: 3.20 ng Dienogest/L Mean measured concentration: 3.51 ng Dienogest/L							
	Total length [cm]															
Replicate	**A**		**B**		**C**		**D**		**A**		**B**		**C**		**D**	
Fish No.	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
1	3.3	-	-	3.7	-	4.2	-	3.5	3.8	-	3.2	-	-	3.8	3.3	-
2	3.9	-	-	3.6	-	3.6	4.0	-	3.7	-	-	3.8	4.2	-	4.1	-
3	3.9	-	-	3.6	-	3.1	-	3.7	-	3.7	-	3.5	4.1	-	4.0	-
4	3.8	-	-	3.5	4.0	-	-	3.6	-	3.8	-	3.7	3.9	-	4.1	-
5	-	3.5	-	3.7	4.1	-	3.6	-	3.8	-	3.5	-	4.0	-	4.0	-
6	3.8	-	3.8	-	4.0	-	3.7	-	-	3.8	-	3.8	4.0	-	-	3.5
7	3.7	-	3.6	-	3.9	-	3.7	-	-	4.1	-	3.7	3.5	-	-	3.5
8	-	3.6	3.7	-	3.9	-	3.7	-	3.8	-	-	3.6	-	3.7	3.8	-
9	-	3.6	3.6	-	3.9	-	-	3.7	-	3.5	-	3.9	-	3.2	-	4.0
10	3.5	-	3.6	-	3.5	-	-	3.8	3.6	-	-	3.9	-	4.0	-	3.6
11	3.7	-	-	3.7	3.6	-	-	3.8	-	3.2	-	3.6	4.2	-	-	3.5
12	3.7	-	3.8	-	-	3.8	3.7	-	3.6	-	-	3.7	4.0	-	-	4.3
13	-	3.5	-	3.6	-	3.5	3.9	-	3.8	-	3.5	-	3.9	-	3.8	-
14	4.0	-	-	3.5	3.8	-	-	3.6	-	3.5	3.5	-	-	3.8	-	3.9
15	-	3.6	-	3.8		4.1	3.8	-	3.7	-	3.6	-	-	3.4	-	3.8

	Control								**Nominal concentration: 3.20 ng Dienogest/L Mean measured concentration: 3.51 ng Dienogest/L**							
16	-	3.2	-	3.5	3.8	-	3.6	-	3.7	-	3.8	-	3.5	-	4.0	-
17	-	3.5	4.0	-	4.0	-	3.3	-	3.7	-	3.8	-	3.7	-		3.5
18	3.6	-	3.5	-	4.0	-	3.7	-	-	3.5	3.7	-	3.9	-	3.8	-
19	3.6	-	-	3.5	3.8	-	3.6	-	-	3.6	3.6	-	3.8	-	4.0	-
20	3.6	-	3.8	-	-	-	3.3	-	3.6	-	-	-	3.8	-	3.6	-
Mean	**3.7**	**3.5**	**3.7**	**3.6**	**3.9**	**3.7**	**3.7**	**3.7**	**3.7**	**3.6**	**3.6**	**3.7**	**3.9**	**3.7**	**3.9**	**3.7**
SD	0.2	0.1	0.2	0.1	0.2	0.4	0.2	0.1	0.1	0.3	0.2	0.1	0.2	0.3	0.2	0.3
RSD	5.1	4.0	4.1	2.9	4.4	11.0	5.4	3.0	2.2	7.0	5.2	3.5	5.7	8.1	6.3	7.7

Table 58: F_1 generation, adult stage: individual total length [cm]; 10.0 and 32.0 ng Dienogest/L, respective 10.3 and 31.7 ng Dienogest/L (mean measured)

	Nominal concentration: 10.0 ng Dienogest/L Mean measured concentration: 10.3 ng Dienogest/L								Nominal concentration: 32.0 ng Dienogest/L Mean measured concentration: 31.7 ng Dienogest/L							
	Total length [cm]															
Replicate	A		B		C		D		A		B		C		D	
Fish No.	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	-	4.0		3.6	-	3.5	-	3.8	3.7	-	-	3.8	-	3.5	-	3.9
2	-	3.8	3.6		3.6	-	-	4.0	-	3.6	-	3.8	-	3.8	3.8	-
3	-	3.8	4.0		-	3.7	-	3.7	4.1	-	-	3.8	-	3.6	-	3.7
4	-	3.8		3.5	3.6	-	-	3.8	-	3.7	3.9	-	3.5	-	-	3.8
5	-	3.9	3.5		3.9	-	-	4.0	4.0	-	4.0	-	-	3.4	3.7	-
6	3.8	-	3.6		3.8	-	-	4.0	-	3.5	-	3.5	-	3.6	3.8	-
7	4.0	-		3.5	3.8	-	-	4.2	3.7	-	3.8	-	3.9	-	-	3.5
8	-	3.3		3.5	3.9	-	3.8	-	4.0	-	3.7	-	3.7	-	3.6	-
9	-	3.6		3.8	-	3.8	4.0	-	-	4.1	3.6	-	3.7	-	-	3.8
10	-	4.0		3.6	-	4.0	3.5	-	3.8	-	4.0	-	-	3.7	-	3.7
11	-	3.8	3.9		-	4.0	3.7	-	-	3.4	-	3.9	-	3.7	-	3.8
12	3.6	-	4.0		-	3.7	-	3.9	3.7	-	3.7	-	-	3.8	-	3.7
13	-	3.5		3.8	4.0	-	3.7	-	3.6	-	4.0	-	-	3.8	-	3.7
14	-	3.7		3.6	3.8	-	4.0	-	3.8	-	4.0	-	3.8	-	-	3.7
15	3.5	-	3.6		-	3.1	-	4.0	-	3.7	3.8	-	-	3.5	-	4.0

	Nominal concentration: 10.0 ng Dienogest/L Mean measured concentration: 10.3 ng Dienogest/L								**Nominal concentration: 32.0 ng Dienogest/L Mean measured concentration: 31.7 ng Dienogest/L**							
16	3.7	-		3.7	4.0	-	3.7	-	-	3.7	3.6	-	-	3.9	-	3.7
17	-	4.2	4.2		3.4	-	-	3.8	4.0	-	-	-	-	3.9	3.8	-
18	-	3.6	3.8		-	3.7	-	3.8	-	3.8	-	-	3.7	-	3.7	-
19	-	3.7		3.3	3.3	-	3.8	-	4.0	-	-	-	3.7	-	3.6	-
20	3.7	-		3.8	3.8	-	-	3.2	3.8	-	-	-		-	3.8	-
Mean	**3.7**	**3.8**	**3.8**	**3.6**	**3.7**	**3.7**	**3.8**	**3.9**	**3.9**	**3.7**	**3.8**	**3.8**	**3.7**	**3.7**	**3.7**	**3.8**
SD	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.1
RSD	4.6	6.0	6.3	4.4	6.0	7.9	4.4	6.4	4.2	5.7	4.2	4.0	3.3	4.5	2.4	3.3

Table 59: F$_1$ generation, adult stage: individual total length [cm]; 100 and 320 ng Dienogest/L, respective 105 and 335 ng Dienogest/L (mean measured)

	Nominal concentration: 100 ng Dienogest/L Mean measured concentration: 105 ng Dienogest/L								Nominal concentration: 320 ng Dienogest/L Mean measured concentration: 335 ng Dienogest/L							
	Total length [cm]															
Replicate	A		B		C		D		A		B		C		D	
Fish No.	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	-	-	-	3.6	-	4.5	-	3.9	-	4.0	-	4.1	-	3.8	-	3.8
2	-	-	-	3.9	-	4.4	-	4.0	-	3.8	-	4.2	-	3.5	3.9	-
3	-	-	-	3.7	-	4.6	-	3.7	-	3.8	-	4.0	3.6	-	-	3.6
4	-	-	-	3.7	4.5	-	3.8	-	4.0		-	4.0	-	4.0	4.0	-
5	-	-	4.0	-	4.3	-	-	4.0	-	4.0	-	3.9	3.5	-	-	3.5
6	-	-	4.0	-	4.5	-	3.7	-	4.1	-	-	3.8	3.4	-	-	3.8
7	-	-	3.8	-	4.1	-	-	3.7	4.1	-	3.9	-	3.7	-	-	3.0
8	-	-	-	3.7	-	-	-	4.3	4.0	-	3.7	-	3.6	-	3.8	-
9	-	-	-	3.5	-	-	-	3.7	3.6	-	4.0	-	4.0	-	3.8	-
10	-	-	-	3.5	-	-	-	3.8	4.1	-	-	-	3.9	-	3.7	-
11	-	-	-	3.6	-	-	3.5	-	3.9	-	-	-	3.6	-	3.6	-
12	-	-	-	3.7	-	-	3.5	-	-	-	-	-	3.5	-	-	4.0
13	-	-	3.4	-	-	-	-	3.8	-	-	-	-	-	4.0	-	3.6
14	-	-	3.9	-	-	-	-	3.7	-	-	-	-	-	3.8	3.8	-
15	-	-	4.0	-	-	-	-	3.6	-	-	-	-	-	3.6	3.7	-

	Nominal concentration: 100 ng Dienogest/L **Mean measured concentration: 105 ng Dienogest/L**								**Nominal concentration: 320 ng Dienogest/L** **Mean measured concentration: 335 ng Dienogest/L**							
16	-	-	-	3.7	-	-	-	3.5	-	-	-	-	-	3.5	3.6	-
17	-	-	-	3.7	-	-	3.6	-	-	-	-	-	4.0	-	3.7	-
18	-	-	3.8	-	-	-	-	-	-	-	-	-	-	3.6	3.6	-
19	-	-	3.9	-	-	-	-	-	-	-	-	-	3.5	-	-	-
20	-	-		-	-	-	-	-	-	-	-	-	-	3.8	-	-
Mean	**-**	**-**	**3.9**	**3.7**	**4.4**	**4.5**	**3.6**	**3.8**	**4.0**	**3.9**	**3.9**	**4.0**	**3.7**	**3.7**	**3.7**	**3.6**
SD	-	-	0.2	0.1	0.2	0.1	0.1	0.2	0.2	0.1	0.2	0.1	0.2	0.2	0.1	0.3
RSD	-	-	5.2	3.1	4.4	2.2	3.6	5.6	4.5	3.0	4.0	3.5	5.8	5.2	3.5	8.8

Table 60: F_1 generation, adult stage: individual wet weight [g]; control and 3.20 ng Dienogest/L (nominal), respective 3.51 ng Dienogest/L (mean measured)

	Control								Nominal concentration: 3.20 ng Dienogest/L Mean measured concentration: 3.51 ng Dienogest/L							
	Wet weight [g]															
Replicate	A		B		C		D		A		B		C		D	
Fish No.	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	0.343	-	-	0.596	-	0.687	-	0.500	0.505	-	0.288	-	-	0.605	0.427	-
2	0.496	-	-	0.501	-	0.552	0.467	-	0.460	-	-	0.578	0.607	-	0.551	-
3	0.481	-	-	0.513	-	0.323	-	0.586	-	0.500	-	0.473	0.547	-	0.513	-
4	0.448	-	-	0.473	0.511	-	-	0.426	-	0.535	-	0.585	0.547	-	0.537	-
5	-	0.486	-	0.507	0.577	-	0.357	-	0.495	-	0.441	-	0.542	-	0.529	-
6	0.429	-	0.415	-	0.575	-	0.416	-	-	0.504	-	0.607	0.461	-	-	0.491
7	0.384	-	0.375	-	0.523	-	0.529	-	-	0.893	-	0.550	0.368	-	-	0.362
8	-	0.481	0.393	-	0.490	-	0.411	-	0.455	-	-	0.500	-	0.527	0.484	-
9	-	0.477	0.354	-	0.422	-	-	0.533	-	0.420	-	0.644	-	0.372	-	0.656
10	0.313	-	0.390	-	0.380	-	-	0.597	0.434	-	-	0.545	-	0.703	-	0.478
11	0.399	-	-	0.472	0.269	-	-	0.612	-	0.374	-	0.532	0.648	-	-	0.444
12	0.379	-	0.474	-	-	0.629	0.412	-	0.422	-	-	0.551	0.493	-	-	0.792
13	-	0.499	-	0.477	-	0.476	0.498	-	0.410	-	0.417	-	0.486	-	0.498	-
14	0.545	-	-	0.481	0.524	-	-	0.447	-	0.439	0.429	-	-	0.596	-	0.606
15	-	0.359	-	0.616	-	0.844	0.485	-	0.432	-	0.374	-	-	0.364	-	0.545

	Control								**Nominal concentration: 3.20 ng Dienogest/L** **Mean measured concentration: 3.51 ng Dienogest/L**							
16	-	0.364	-	0.440	0.442	-	0.432	-	0.469	-	0.380	-	0.338	-	0.531	-
17	-	0.364	0.474	-	0.511	-	0.410	-	0.460	-	0.457	-	0.437	-	-	0.378
18	0.458	-	0.404	-	0.480	-	0.416	-	-	0.511	0.424	-	0.544	-	0.464	-
19	0.414	-	-	0.454	0.441	-	0.459	-	-	0.550	0.417	-	0.434	-	0.492	-
20	0.469	-	0.409	-	-	-	0.332	-	0.412	-		-	0.505	-	0.388	-
Mean	**0.428**	**0.433**	**0.410**	**0.503**	**0.473**	**0.585**	**0.433**	**0.529**	**0.450**	**0.525**	**0.403**	**0.557**	**0.497**	**0.528**	**0.492**	**0.528**
SD	0.065	0.066	0.041	0.056	0.084	0.179	0.055	0.074	0.032	0.149	0.051	0.050	0.086	0.136	0.050	0.138
RSD	15.1	15.3	10.0	11.1	17.7	30.7	12.7	14.0	7.0	28.5	12.6	9.0	17.2	25.8	10.1	26.2

Table 61: F_1 generation, adult stage: individual wet weight [g]; 10.0 and 32.0 ng Dienogest/L, respective 10.3 and 31.7 ng Dienogest/L (mean measured)

	Nominal concentration: 10.0 ng Dienogest/L Mean measured concentration: 10.3 ng Dienogest/L								Nominal concentration: 32.0 ng Dienogest/L Mean measured concentration: 31.7 ng Dienogest/L							
	Wet weight [g]															
Replicate	**A**		**B**		**C**		**D**		**A**		**B**		**C**		**D**	
Fish No.	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
1	-	0.629	-	0.625	-	0.409	-	0.582	0.464	-	-	0.624	-	0.522	-	0.567
2	-	0.664	0.394	-	0.406	-	-	0.732	-	0.623	-	0.729	-	0.555	0.418	-
3	-	0.680	0.526	-	-	0.574	-	0.548	0.604	-	-	0.632	-	0.470	-	0.520
4	-	0.598	-	0.388	0.465	-	-	0.565	-	0.548	0.403	-	0.402	-	-	0.578
5	-	0.690	0.306	-	0.463	-	-	0.651	0.452	-	0.464	-		0.390	0.348	-
6	0.501	-	0.353	-	0.458	-	-	0.640	-	0.456	-	0.419	-	0.464	0.371	-
7	0.479	-	-	0.523	0.425	-	-	0.884	0.355	-	0.500	-	0.499	-	-	0.453
8	-	0.364	-	0.557	0.452	-	0.430	-	0.557	-	0.393	-	0.430	-	0.351	-
9	-	0.586	-	0.701	-	0.628	0.501	-		0.787	0.409	-	0.422	-	-	0.520
10	-	0.628	-	0.527	-	0.667	0.379	-	0.457	-	0.571	-	-	0.579	-	0.484
11	-	0.508	0.509	-	-	0.641	0.514	-	-	0.384	-	0.792	-	0.539	-	0.555
12	0.421	-	0.548	-	-	0.575	-	0.534	0.461	-	0.425	-	-	0.560	-	0.482
13	-	0.460	-	0.602	0.532	-	0.379	-	0.472	-	0.538	-	-	0.540	-	0.479
14	-	0.570	-	0.556	0.431	-	0.550	-	0.443	-	0.523	-	0.476	-	-	0.460
15	0.379	-	0.418	-	-	0.369	-	0.600	-	0.434	0.459	-	-	0.434	-	0.595

	Nominal concentration: 10.0 ng Dienogest/L Mean measured concentration: 10.3 ng Dienogest/L								**Nominal concentration: 32.0 ng Dienogest/L Mean measured concentration: 31.7 ng Dienogest/L**							
16	0.465	-	-	0.424	0.507	-	0.402	-	-	0.487	0.439	-	-	0.592	-	0.449
17	-	0.722	0.583	-	0.358	-	-	0.514	0.553		-	-	-	0.578	0.415	-
18	-	0.574	0.491	-	-	0.546	-	0.539	-	0.492	-	-	0.460	-	0.393	-
19	-	0.547	-	0.422	0.294	-	0.468	-	0.549	-	-	-	0.463	-	0.344	-
20	0.449	-	-	0.535	0.423	-	-	0.261	0.423	-	-	-	-	-	0.421	-
Mean	**0.449**	**0.587**	**0.459**	**0.533**	**0.435**	**0.551**	**0.453**	**0.588**	**0.483**	**0.526**	**0.466**	**0.639**	**0.450**	**0.519**	**0.383**	**0.512**
SD	0.044	0.097	0.095	0.094	0.063	0.108	0.065	0.146	0.070	0.128	0.060	0.142	0.034	0.064	0.033	0.051
RSD	9.7	16.4	20.7	17.6	14.6	19.6	14.4	24.9	14.5	24.3	12.8	22.2	7.5	12.4	8.7	10.1

Table 62: F_1 generation, adult stage: individual wet weight [g]; 100 and 320 ng Dienogest/L; respective 105 and 335 ng Dienogest/L (mean measured)

	Nominal concentration: 100 ng Dienogest/L Mean measured concentration: 105 ng Dienogest/L								Nominal concentration: 320 ng Dienogest/L Mean measured concentration: 335 ng Dienogest/L							
	Wet weight [g]															
Replicate	A		B		C		D		A		B		C		D	
Fish No.	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	-	-	-	0.475	-	0.977	-	0.553	-	0.760	-	0.785	-	0.596	-	0.669
2	-	-	-	0.741	-	0.936	-	0.573	-	0.646	-	0.920	-	0.486	0.556	-
3	-	-	-	0.492	-	1.171	-	0.537	-	0.537	-	0.722	0.414	-	-	0.652
4	-	-	-	0.573	0.833	-	0.422	-	0.576		-	0.824	-	0.555	0.522	
5	-	-	0.533	-	0.616	-	-	0.600		0.773	-	0.570	0.432	-	-	0.529
6	-	-	0.491	-	0.774	-	0.390	-	0.688	-	-	0.719	0.326	-	-	0.646
7	-	-	0.442	-	0.540	-	-	0.516	0.555	-	0.503	-	0.482	-	-	0.315
8	-	-	-	0.555	-	-	-	0.374	0.559	-	0.491	-	0.469	-	0.520	-
9	-	-	-	0.423	-	-	-	0.564	0.445	-	0.547	-	0.460	-	0.465	-
10	-	-	-	0.550	-	-	-	0.570	0.600	-	-	-	0.502	-	0.440	-
11	-	-	-	0.533	-	-	0.317	-	0.520	-	-	-	0.410	-	0.390	-
12	-	-	-	0.527	-	-	0.326	-	-	-	-	-	0.339	-	-	0.700
13	-	-	0.471	-	-	-	-	0.564	-	-	-	-	-	0.946	-	0.480
14	-	-	0.500	-	-	-	-	0.599	-	-	-	-	-	0.596	0.530	-
15	-	-	0.480	-	-	-	-	0.497	-	-	-	-	-	0.520	0.485	-

	Nominal concentration: 100 ng Dienogest/L **Mean measured concentration: 105 ng Dienogest/L**								**Nominal concentration: 320 ng Dienogest/L** **Mean measured concentration: 335 ng Dienogest/L**							
16	-	-	-	0.563	-	-	-	0.432	-	-	-	-	-	0.442	0.421	-
17	-	-	-	0.523	-	-	0.422	-	-	-	-	-	0.553	-	0.480	-
18	-	-	0.442	-	-	-	-	-	-	-	-	-	-	0.563	0.384	-
19	-	-	0.487	-	-	-	-	-	-	-	-	-	0.360	-	-	-
20	-	-	-	-	-	-	-	-	-	-	-	-	-	0.558	-	-
Mean	**-**	**-**	**0.481**	**0.541**	**0.691**	**1.028**	**0.375**	**0.532**	**0.563**	**0.679**	**0.514**	**0.757**	**0.432**	**0.585**	**0.472**	**0.570**
SD	-	-	0.030	0.079	0.136	0.126	0.051	0.068	0.074	0.111	0.029	0.118	0.071	0.145	0.058	0.138
RSD	-	-	6.3	14.7	19.7	12.2	13.6	12.8	13.2	16.3	5.7	15.6	16.4	24.7	12.3	24.2

Table 63: F_1 generation, adult stage, summary: mean total length and mean wet weight

		Nominal concentration Dienogest [ng/L]					
		Control	3.20	10.0	32.0	100	320
		Mean measured concentration Dienogest [ng/L]					
	Replicate	Control	3.51	10.3	31.7	105	335
Total length males, adult stage [cm]	A	3.7	3.7	3.7	3.9		4.0
	B	3.7	3.6	3.8	3.8	3.9	3.9
	C	3.9	3.9	3.7	3.7	4.4	3.7
	D	3.7	3.9	3.8	3.7	3.6	3.7
	Mean	**3.7**	**3.8**	**3.8**	**3.8**	**3.9**	**3.8**
	SD	0.1	0.1	0.0	0.1	0.4	0.1
	RSD	2.5	3.9	1.0	1.8	9.5	3.5
Total length females, adult stage [cm]	A	3.5	3.6	3.8	3.7		3.9
	B	3.6	3.7	3.6	3.8	3.7	4.0
	C	3.7	3.7	3.7	3.7	4.5	3.7
	D	3.7	3.7	3.9	3.8	3.8	3.6
	Mean	**3.6**	**3.7**	**3.7**	**3.7**	**4.0**	**3.8**
	SD	0.1	0.0	0.1	0.0	0.4	0.2
	RSD	2.6	1.4	2.8	1.1	11.2	4.5
Wet weight males, adult stage [g]	A	0.428	0.450	0.449	0.483	-	0.563
	B	0.410	0.403	0.459	0.466	0.481	0.514
	C	0.473	0.497	0.435	0.450	0.691	0.432
	D	0.433	0.492	0.453	0.383	0.375	0.472
	Mean	**0.436**	**0.461**	**0.449**	**0.445**	**0.516**	**0.495**
	SD	0.027	0.044	0.010	0.044	0.161	0.056
	RSD	6.1	9.5	2.3	9.8	31.1	11.4
Wet weight females, adult stage [g]	A	0.433	0.525	0.587	0.526	-	0.679
	B	0.503	0.557	0.533	0.639	0.541	0.757
	C	0.585	0.528	0.551	0.519	1.028	0.585
	D	0.529	0.528	0.588	0.512	0.532	0.570
	Mean	**0.512**	**0.534**	**0.565**	**0.549**	**0.700**	**0.648**
	SD	0.063	0.015	0.027	0.060	0.284	0.087
	RSD	12.3	2.8	4.8	11.0	40.5	13.5

Table 64: **F_1 generation, sex ratio, control, 3.20 and 10.0 ng Dienogest/L (nominal), respective 3.51 and 10.3 ng Dienogest/L (mean measured); (based on histological data provided in C.1.14)**

	Nominal concentration Dienogest [ng/L]											
	Control				3.20				10.0			
	Mean measured concentration Dienogest [ng/L]											
	Control				3.51				10.3			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Male [n]	13	9	13	13	11	9	14	11	6	9	12	8
Female [n]	7	11	6	7	9	10	6	9	14	11	8	12
Total [n]	20	20	19	20	20	19	20	20	20	20	20	20
Male [%]	65.0	45.0	68.4	65.0	55.0	47.4	70.0	55.0	30.0	45.0	60.0	40.0
Female [%]	35.0	55.0	31.6	35.0	45.0	52.6	30.0	45.0	70.0	55.0	40.0	60.0
Total [%]	100	100	100	100	100	100	100	100	100	100	100	100
Total mean male [%]				**60.9**				**56.8**				**43.8**
SD				10.7				9.5				12.5
RSD				17.6				16.7				28.6
Total mean female [%]				**39.1**				**43.2**				**56.3**
SD				10.7				9.5				12.5
RSD				27.3				22.0				22.2

Table 65: F_1 generation, sex ratio, 32.0, 100 and 320 ng Dienogest/L (nominal), respective 31.7, 105 and 335 ng Dienogest/L (mean measured); (based on histological data provided in C.1.14)

	Nominal concentration Dienogest [ng/L]											
	32.0				100				320			
	Mean measured concentration Dienogest [ng/L]											
	31.7				105				335			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Male [n]	12	11	7	8	-	8	4	5	7	3	11	11
Female [n]	8	5	12	12	-	11	3	12	4	6	9	7
Total [n]	20	16	19	20	-	19	7	17	11	9	20	18
Male [%]	60.0	68.8	36.8	40.0	-	42.1	57.1	29.4	63.6	33.3	55.0	61.1
Female [%]	40.0	31.3	63.2	60.0	-	57.9	42.9	70.6	36.4	66.7	45.0	38.9
Total [%]	100	100	100	100	-	100	100	100	100	100	100	100
Total mean male [%]				**51.4**				**42.9**				**53.3**
SD				15.5				13.9				13.8
RSD				30.1				32.4				25.9
Total mean female [%]				**48.6**				**57.1**				**46.7**
SD				15.5				13.9				13.8
RSD				31.8				24.3				29.5

C.1.10 Second filial generation (F_2)

Table 66: Second filial generation (F_2), hatching success

		Nominal concentration Dienogest [ng/L]					
		Control	3.20	10.0	32.0	100	320
		Mean measured concentration Dienogest [ng/L]					
	Replicate	Control	3.51	10.3	31.7	105	335
Introduced eggs [n]	A	20	20	20	20	-	20
	B	20	20	20	20	20	20
	C	20	20	20	20	20	20
	D	20	20	20	20	20	20
Coagulated eggs [n]	A	5	2	3	10	-	19
	B	4	4	16	13	11	14
	C	0	7	10	13	7	10
	D	4	7	8	7	8	5
Hatch, day 4 pf [n]	A	15	18	17	10	-	1
	B	16	16	4	7	9	6
	C	20	13	10	7	13	10
	D	16	13	12	13	12	15
Hatch, day 4 pf [%]	A	75.0	90.0	85.0	50.0	-	5.0
	B	80.0	80.0	20.0	35.0	45.0	30.0
	C	100	65.0	50.0	35.0	65.0	50.0
	D	80.0	65.0	60.0	65.0	60.0	75.0
	Mean	**83.8**	**75.0**	**53.8(*)**	**46.3(*)**	**56.7(*)**	**40.0(*)**
	SD	11.1	12.2	26.9	14.4	10.4	29.7
	RSD	13.2	16.3	50.0	31.1	18.4	74.3

(*) Statistically significant compared to control, $p<0.05$, Williams test, one-sided smaller.

C.1.11 Biomarker evaluation, F_0 generation

Table 67: F_0 generation, Vitellogenin content [ng/mL], control

VTG [ng/mL]	control							
Replicate	**A**		**B**		**C**		**D**	
Fish No	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**
1		1.64E+07	54.77			7.92E+07	47.9	
2	66.67			2.72E+07	49.42			5.86E+07
3		5.98E+07	62.56			1.22E+07	26.91	
4	46.38		101.1			3.31E+07	33.47	
5	55.9			2.04E+07	38.49			3.03E+07
6		1.59E+07		1.54E+07		4.15E+06		2.32E+07
7	41.18		31.32		44.98			2.21E+06
8		9.59E+06		1.18E+07		2.41E+06		1.65E+07
9	58.22		521.6		237.1		45.99	
10		1.07E+07		2.09E+07			44.21	
Geomean	**52.90**	**1.74E+07**	**89.24**	**1.84E+07**	**67.11**	**1.26E+07**	**38.78**	**1.72E+07**

Table 68: F_0 generation, Vitellogenin content [ng/mL], 3.20 ng Dienogest/L (nominal), respective 3.51 ng Dienogest/L (mean measured)

VTG [ng/mL]	3.20 ng Dienogest/L (nominal), respective 3.51 ng Dienogest/L (mean measured)							
Replicate	**A**		**B**		**C**		**D**	
Fish No	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**
1		3.73E+07	137.4			4.27E+07		5.13E+07
2		3.64E+07		5.77E+07	2077			2.50E+07
3		2.10E+07		2.74E+07		1.46E+07		2.76E+07
4	19800		42.44			4.66E+07	302.8	
5	91.96			1.71E+07		1.14E+07		3.81E+07
6	69.13		41.65		404.5			2.58E+07
7	39.91			2.51E+07		1.60E+07	1017	
8		1.66E+07		1.91E+06	5912		1192	
9	41.43		193		88.44		3224	
10	67.25			6.58E+05	92.3			1.52E+06
Geomean	**155.24**	**2.62E+07**	**82.74**	**9.74E+06**	**526.73**	**2.21E+07**	**1043.01**	**1.94E+07**

Table 69: F_0 generation, Vitellogenin content [ng/mL], 10.0 ng Dienogest/L (nominal), respective 10.3 ng Dienogest/L (mean measured)

VTG [ng/mL]	10.0 ng Dienogest/L (nominal), respective 10.3 ng Dienogest/L (mean measured)							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1		3.23E+07(*)	64.15			1.43E+07	633.4	
2		6.42E+07	33.51		81.36			2.79E+07
3		1.66E+07		1.43E+07		8.26E+06	2054	
4	53.66			1.90E+07		1.20E+07	46.76	
5		5.84E+07		5.64E+07	232.8		70.3	
6		3.35E+07		4.28E+07		3.39E+07	144.6	
7	672.2			3.46E+07	86.75			1.31E+07
8	1170		90.93			2.67E+07		8.00E+07
9	2770		268.5			1.07E+07		1.47E+07
10	72.87		471.7		52.38			2.23E+07
Geomean	**385.54**	**3.80E+07**	**119.88**	**2.96E+07**	**96.32**	**1.55E+07**	**228.17**	**2.49E+07**

(*) The hermaphrodite fish was excluded from calculation of the mean.

Table 70: F_0 generation, Vitellogenin content [ng/mL], 32.0 ng Dienogest/L (nominal), respective 31.7 ng Dienogest/L (mean measured)

VTG [ng/mL]	32.0 ng Dienogest/L (nominal), respective 31.7 ng Dienogest/L (mean measured)							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1		2.98E+07	135			3.34E+07		2.58E+07
2		1.56E+07		1.69E+07		1.34E+07	51.1	
3		1.44E+07	262.5			3.91E+07		2.40E+07
4		1.16E+07		2.75E+07	191.5			3.29E+07
5	65.69		434000			5.70E+07	42.3	
6	885			8.58E+06	54.29			1.81E+07
7		2.32E+07		9.43E+06	184.2		1758	
8	89.29		*<10*			2.14E+07	100.9	
9	78.27		60.41		459.2		98.71	
10	85.73			2.84E+07	73.75			
Geomean	**128.35**	**1.78E+07**	**981.78**	**1.61E+07**	**145.34**	**2.92E+07**	**130.50**	**2.46E+07**

The value highlighted in italics was not included in the calculation of the mean.

Table 71: F_0 generation, Vitellogenin content [ng/mL], 100 ng Dienogest/L (nominal), respective 105 ng Dienogest/L (mean measured)

VTG [ng/mL]	100 ng Dienogest/L (nominal), respective 105 ng Dienogest/L (mean measured)							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1	387.7			1.92E+06		1.78E+07		1.64E+07
2		6.49E+07		2.29E+07		1.70E+07	738.2	
3		1.00E+07		2.72E+07	37.09		79.43	
4		1.61E+07		1.18E+07		1.78E+07		2.69E+07
5		9.08E+06		1.42E+07		1.32E+07	63.6	
6	916.1		55.31		84.41			8.67E+06
7	201.2		370.6			1.00E+07		5.88E+06
8		2.45E+07	61.49		1638		15.95	
9	17.02		692.9		63.99		72.98	
10	141.7		323.5		39.55		50.08	
Geomean	**176.72**	**1.88E+07**	**195.08**	**1.15E+07**	**105.35**	**1.48E+07**	**77.54**	**1.22E+07**

Table 72: F_0 generation, Vitellogenin content [ng/mL], 320 ng Dienogest/L (nominal), respective 335 ng Dienogest/L (mean measured)

VTG [ng/mL]	320 ng Dienogest/L (nominal), respective 335 ng Dienogest/L (mean measured)							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1		3.95E+07		8.55E+06	153.8		351	
2		1.24E+07		1.12E+07		2.78E+07		1.88E+07
3		4.83E+06		7.09E+06	85.86			1.21E+07
4		1.45E+07	41.14			3.11E+07		1.64E+07
5	11262			4.42E+06		1.03E+07		2.94E+07
6		6.68E+06	59.69		46.87		255	
7	273.1		56.47			1.90E+07		1.75E+07
8	4640		37.17		61.46		359.1	
9	356.1		598.4			6.56E+06	2212	
10	54.39		100.6		97.28		185.8	
Geomean	**773.23**	**1.18E+07**	**82.28**	**7.40E+06**	**81.97**	**1.62E+07**	**420.90**	**1.81E+07**

Table 73: F_0 generation, Vitellogenin content [ng/mL], summary

		Nominal concentration Dienogest [ng/L]					
		Control	**3.20**	**10.0**	**32.0**	**100**	**320**
		Mean measured concentration Dienogest [ng/L]					
	Replicate	**Control**	**3.51**	**10.3**	**31.7**	**105**	**335**
VTG males [ng/mL]	A	52.90	155.24	385.54	128.35	176.72	773.23
	B	89.24	82.74	119.88	981.78	195.08	82.28
	C	67.11	526.73	96.32	145.34	105.35	81.97
	D	38.78	1043.01	228.17	130.50	77.54	420.90
	Mean	**62.01**	**451.93**	**207.48**	**346.49**	**138.67**	**339.60**
	SD	21.52	439.43	131.87	423.59	56.20	330.27
	RSD	34.71	97.23	63.56	122.25	40.53	97.25
VTG females [ng/mL]	A	1.74E+07	2.62E+07	3.80E+07	1.78E+07	1.88E+07	1.18E+07
	B	1.84E+07	9.74E+06	2.96E+07	1.61E+07	1.15E+07	7.40E+06
	C	1.26E+07	2.21E+07	1.55E+07	2.92E+07	1.48E+07	1.62E+07
	D	1.72E+07	1.94E+07	2.49E+07	2.46E+07	1.22E+07	1.81E+07
	Mean	**1.64E+07**	**1.94E+07**	**2.70E+07**	**2.19E+07**	**1.43E+07**	**1.34E+07**
	SD	2.58E+06	7.01E+06	9.40E+06	6.11E+06	3.28E+06	4.76E+06
	RSD	1.57E+01	3.62E+01	3.48E+01	2.78E+01	2.29E+01	3.56E+01

C.1.12 Biomarker evaluation, F_1 generation

Table 74: F_1 generation, Vitellogenin content [ng/mL], control

	VTG content [ng/mL] at control							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1	120.4			4.21E+06		3.17E+07		8.78E+06
2	38.79			1.37E+07		2.46E+07	60.95	
3	31.35			2.17E+07		1.08E+07		1.93E+07
4	24.3			1.84E+07	334.8			186.5
5		3.72E+07		1.47E+07	40127		65.5	
6	55.38		137.4		805.8		49.37	
7	61.16		32.76		35.34		39.86	
8		3.36E+07	61.88		67.3		43.6	
9		2.54E+07	56.88		1907			7.34E+07
10	63.24		27.43		46.77			2.14E+07
11	46588			2.68E+07	50.77			1.88E+07
12	54.57		9430000			3.15E+07	1215	
13		4.28E+07		1.28E+07		5.38E+07	47.64	
14	44.16			1.95E+07	135.5			1.60E+07
15		1.47E+07		1.17E+07		4.12E+07	497000	
16		1.72E+07		1.41E+07	1077		102.3	
17		1.53E+07	58.85		174.3		64.28	
18	53.83		27.86		84.32		41.87	
19	33.42			2.53E+07	182.8		116.8	
20	109.5		18.9				646.9	
Geomean	**87.20**	**2.45E+07**	**171.30**	**1.51E+07**	**272.51**	**2.89E+07**	**179.78**	**3.95E+06**

Table 75: F_1 generation, Vitellogenin content [ng/mL], 3.20 ng Dienogest/L (nominal), respective 3.51 ng Dienogest/L (mean measured)

VTG [ng/mL]	3.20 ng Dienogest/L (nominal), respective 3.51 ng Dienogest/L (mean measured)							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1	41.67		13.3			2.06E+07	23.58	
2	50.01			1.16E+07	62.82		30.98	
3		2.16E+07		5.82E+06	51.68		880.4	
4		1.71E+07		1.88E+07	75.83		366.7	
5	34.18		40.84		22		96.9	
6		8.09E+06		7.64E+06	16.04			3.71E+06
7		8.12E+06		9.95E+06	24.76			9.73E+06
8	32.3			8.91E+05		8.64E+06	856.7	
9		1.61E+07		3.49E+07		3.20E+06		3.86E+07
10	53.54			1.78E+07		1.10E+07		3.23E+07
11		5.59E+06		3.97E+07	1315			2.02E+07
12	291.6			1.53E+07	45.23			2.55E+07
13	79.01		6.85		10.09		56.3	
14		2.91E+07	42.23			1.53E+07		4.48E+07
15	41.32		33.92			6.79E+06		4.27E+07
16	27.19		68.38		190		34.12	
17	77.43		47.98		79.06			6.49E+06
18		2.01E+07	82.16		141.3		21.55	
19		3.14E+07	33.11		34.77		59.01	
20	30.72				27.95		17.88	
Geomean	**52.04**	**1.51E+07**	**33.09**	**1.12E+07**	**55.55**	**9.31E+06**	**79.97**	**1.88E+07**

Table 76: F_1 generation, Vitellogenin content [ng/mL], 10.0 ng Dienogest/L (nominal), respective 10.3 ng Dienogest/L (mean measured)

VTG [ng/mL]	10.0 ng Dienogest/L (nominal), respective 10.3 ng Dienogest/L (mean measured)							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1		1.87E+07		2.62E+07		4.77E+06		2.06E+07
2		2.89E+07	81.43		25.05			5.49E+07
3		4.21E+07	40.18			9.64E+06		8.34E+06
4		2.88E+07		2.72E+07	54.58			1.21E+07
5		2.08E+07	36.88		105			6.42E+06
6	378.5		5,782		23.44			1.46E+07
7	218			2.20E+07	60.71			2.58E+07
8		8.07E+06		5.10E+07	66.85		77.61	
9		1.42E+07		2.11E+07		6.05E+07	49.45	
10		5.11E+07		2.44E+07		1.76E+07	18.62	
11		1.31E+07	201.8			1.80E+07	55.16	
12	128.6		65.68			3.35E+06		3.37E+07
13		1.47E+07		5.71E+07	73.88		27.06	
14		1.07E+07		1.32E+07	37.95		47.81	
15	17.81		144.4			2.41E+07		4.63E+07
16	45.84			1.29E+07	14.67		49.27	
17		3.02E+07	91.96		72.3			8.84E+06
18		1.86E+07	57.25			7.22E+06		6.22E+05
19		1.81E+07		9.00E+06	25.05		73.96	
20	393.2			4.15E+07	51.44			8.10E+06
Geomean	**122.66**	**2.00E+07**	**124.20**	**2.40E+07**	**43.98**	**1.23E+07**	**45.57**	**1.28E+07**

Table 77: F_1 generation, Vitellogenin content [ng/mL], 32.0 ng Dienogest/L (nominal), respective 31.7 ng Dienogest/L (mean measured)

VTG [ng/mL]	32.0 ng Dienogest/L (nominal), respective 31.7 ng Dienogest/L (mean measured)							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1	143.2			3.28E+07		6.15E+06		3.74E+07
2		1.57E+07		1.32E+07		3.41E+07	8.02E+05	
3	60.13			7.91E+06		1.33E+07		1.36E+07
4		9.62E+06	25.67		25.68			3.07E+07
5	151.9		204.9			1.19E+07	245.7	
6		4.97E+06		4.17E+07		7.76E+06	133.3	
7	239.6		119.7		38.31			1.46E+07
8	187.2		18.11		74.92		137	
9		5.75E+07	40		92.24			1.10E+07
10	114.2		168.6			7.30E+06		1.60E+07
11		1.59E+07		3.24E+07		2.75E+07		3.02E+07
12	164.5		150.7			1.44E+07		1.35E+07
13	29.46		78.25			1.88E+07		1.29E+07
14	34.08		27.19		46.15			1.01E+07
15		9.39E+06	13.58			2.80E+07		1.13E+07
16		1.03E+07	63.09			2.98E+07		4.47E+06
17	41.32					1.45E+07	82.21	
18		1.23E+07			130.7		417.7	
19	83.14				55.83		2.13E+04	
20	154.6						22.65	
Geomean	**95.67**	**1.30E+07**	**57.12**	**2.15E+07**	**58.30**	**1.53E+07**	**702.85**	**1.48E+07**

Table 78: F_1 generation, Vitellogenin content [ng/mL], 100 ng Dienogest/L (nominal), respective 105 ng Dienogest/L (mean measured)

VTG [ng/mL]	100 ng Dienogest/L (nominal), respective 105 ng Dienogest/L (mean measured)							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1	-	-		3.78E+07		5.78E+07		3.51E+07
2	-	-		3.57E+07		7.49E+07		4.86E+07
3	-	-		9.06E+06		5.37E+07		6.30E+07
4	-	-		1.00E+07	1128		1054	
5	-	-	1776		36.34			1.21E+08
6	-	-	637.9		43.05		156.1	
7	-	-	151		90.72			6.44E+07
8	-	-		2.71E+07				3.45E+07
9	-	-		6.20E+06				1.25E+07
10	-	-		2.48E+07				1.43E+07
11	-	-		6.00E+06			3.0E+06	
12	-	-		1.70E+07			5.5E+05	
13	-	-	2265					1.72E+07
14	-	-	1437					2.12E+07
15	-	-	2360					1.83E+07
16	-	-		1.90E+07				2.12E+07
17	-	-		2.14E+07			3.9E+05	
18	-	-	171.5					
19	-	-	101.9					
20	-	-						
Geomean	**-**	**-**	**623.92**	**1.63E+07**	**112.48**	**6.15E+07**	**40178.87**	**3.08E+07**

Table 79: F_1 generation, Vitellogenin content [ng/mL], 320 ng Dienogest/L (nominal), respective 335 ng Dienogest/L (mean measured)

VTG [ng/mL]	320 ng Dienogest/L (nominal), respective 335 ng Dienogest/L (mean measured)							
Replicate	**A**		**B**		**C**		**D**	
Fish No	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**
1		3.76E+07		3.80E+07		2.73E+07		8.41E+07
2		1.43E+07		5.17E+07		7.27E+06	247.8	
3		3.49E+07		3.90E+07	67.53			2.29E+07
4	21.09			*>1.60E+08*		2.35E+07	66	
5		6.21E+07		1.71E+07	1605			4.46E+07
6	34.43			4.08E+07	207.8			4.24E+07
7	35.65		93.01		1157			1.39E+07
8	46.73		5334		108.5		162.1	
9	21.55		774.5		153.7		322	
10	42.73				128.8		1636	
11	60.93				172		158.7	
12					21.03			7.20E+07
13						2.61E+07		1.08E+07
14						2.50E+07	260.4	
15						4.17E+07	489.6	
16						7.20E+06	144	
17					762.9		235.7	
18						7.07E+06	275.4	
19					17.2			
20						1.43E+07		
Geomean	**35.27**	**3.29E+07**	**727.00**	**3.51E+07**	**166.11**	**1.65E+07**	**252.34**	**3.26E+07**

The value highlighted in *italics* was not included in the calculation of the mean

Table 80: F_1 generation, Vitellogenin content [ng/mL], summary

		Nominal concentration Dienogest [ng/L]					
		Control	**3.20**	**10.0**	**32.0**	**100**	**320**
		Mean measured concentration Dienogest [ng/L]					
	Replicate	**Control**	**3.51**	**10.3**	**31.7**	**105**	**335**
VTG males [ng/mL]	A	87.20	52.04	122.66	95.67	-	35.27
	B	171.30	33.09	124.20	57.12	623.92	727.00
	C	272.51	55.55	43.98	58.30	112.48	166.11
	D	179.78	79.97	45.57	702.85	40178.87	252.34
	Mean	**177.70**	**55.16**	**84.10**	**228.48**	**13638.42**	**295.18**
	SD	75.77	19.26	45.42	316.75	22986.12	301.40
	RSD	42.64	34.91	54.01	138.63	168.54	102.11
VTG females [ng/mL]	A	2.45E+07	1.51E+07	2.00E+07	1.30E+07	-	3.29E+07
	B	1.51E+07	1.12E+07	2.40E+07	2.15E+07	1.63E+07	3.51E+07
	C	2.89E+07	9.31E+06	1.23E+07	1.53E+07	6.15E+07	1.65E+07
	D	3.95E+06	1.88E+07	1.28E+07	1.48E+07	3.08E+07	3.26E+07
	Mean	**1.81E+07**	**1.36E+07**	**1.73E+07**	**1.62E+07**	**3.62E+07**	**2.93E+07**
	SD	1.11E+07	4.19E+06	5.70E+06	3.72E+06	2.31E+07	8.57E+06
	RSD	61.07	30.83	33.02	23.00	63.79	29.28

C.1.13 Histopathology of fish gonads, F_0 generation

Table 81: Histopathology raw data, F_0 generation

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage
control	1	P-ZF1	P-0/1	f	f		3	1				
control	2	P-ZF2	P-0/1	m	m							2
control	3	P-ZF3	P-0/1	f	f		3					
control	4	P-ZF4	P-0/1	m	m							2
control	5	P-ZF5	P-0/1	m	m							2
control	6	P-ZF6	P-0/1	f	f		3					
control	7	P-ZF7	P-0/1	m	m							2
control	8	P-ZF8	P-0/1	f	f		3					
control	9	P-ZF9	P-0/1	m	m							2
control	10	P-ZF10	P-0/1	f	f		3	1			1	
control	1	P-ZF11	P-0/2	m	m							2
control	2	P-ZF12	P-0/2	f	f		3	1	1	1		
control	3	P-ZF13	P-0/2	m	m							2
control	4	P-ZF14	P-0/2	m	m							2

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage
control	5	P-ZF15	P-0/2	f	f		3		1		2	
control	6	P-ZF16	P-0/2	f	f		2	2				
control	7	P-ZF17	P-0/2	m	m							2
control	8	P-ZF18	P-0/2	f	f		3					
control	9	P-ZF19	P-0/2	m	m							2
control	10	P-ZF20	P-0/2	f	f		2				1	
control	1	P-ZF21	P-0/3	f	f		4	1		2		
control	2	P-ZF22	P-0/3	m	m							2
control	3	P-ZF23	P-0/3	f	f		2					
control	4	P-ZF24	P-0/3	f	f		3					
control	5	P-ZF25	P-0/3	m	m							1
control	6	P-ZF26	P-0/3	f	f		3					
control	7	P-ZF27	P-0/3	m	m							2
control	8	P-ZF28	P-0/3	f	f		2				2	
control	9	P-ZF29	P-0/3	m	m							2
control	1	P-ZF30	P-0/4	m	m							1
control	2	P-ZF31	P-0/4	f	f		3				1	

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage
control	3	P-ZF32	P-0/4	m	m							2
control	4	P-ZF33	P-0/4	m	m							2
control	5	P-ZF34	P-0/4	f	f		4					
control	6	P-ZF35	P-0/4	f	f		3			1		
control	7	P-ZF36	P-0/4	m	f		2					
control	8	P-ZF37	P-0/4	f	f		3					
control	9	P-ZF38	P-0/4	m	m							2
control	10	P-ZF39	P-0/4	m	m							3
3.20	1	P-ZF40	P-1/1	f	f		3	2	2			
3.20	2	P-ZF41	P-1/1	f	f		2					
3.20	3	P-ZF42	P-1/1	f	f		3		2		1	
3.20	4	P-ZF43	P-1/1	m	m							2
3.20	5	P-ZF44	P-1/1	m	m							3
3.20	6	P-ZF45	P-1/1	m	m							2
3.20	7	P-ZF46	P-1/1	m	m							2
3.20	8	P-ZF47	P-1/1	f	f		2				1	
3.20	9	P-ZF48	P-1/1	m	m							2

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage
3.20	10	P-ZF49	P-1/1	m	m							3
3.20	1	P-ZF50	P-1/2	m	m							2
3.20	2	P-ZF51	P-1/2	f	f		2	1				
3.20	3	P-ZF52	P-1/2	f	f		3					
3.20	4	P-ZF53	P-1/2	m	m							2
3.20	5	P-ZF54	P-1/2	f	f		2	1	2			
3.20	6	P-ZF55	P-1/2	m	m							2
3.20	7	P-ZF56	P-1/2	f	f		2		2			
3.20	8	P-ZF57	P-1/2	f	f		2					
3.20	9	P-ZF58	P-1/2	m	m							2
3.20	10	P-ZF59	P-1/2	f	f		1					
3.20	1	P-ZF60	P-1/3	f	f		3					
3.20	2	P-ZF61	P-1/3	m	m							2
3.20	3	P-ZF62	P-1/3	f	f		3					
3.20	4	P-ZF63	P-1/3	f	f		3					
3.20	5	P-ZF64	P-1/3	f	f		2		2		1	
3.20	6	P-ZF65	P-1/3	m	m							2

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage
3.20	7	P-ZF66	P-1/3	f	f		2		1		1	
3.20	8	P-ZF67	P-1/3	m	m							2
3.20	9	P-ZF68	P-1/3	m	m							2
3.20	10	P-ZF69	P-1/3	m	m							3
3.20	1	P-ZF70	P-1/4	f	f		3	1	2	1		
3.20	2	P-ZF71	P-1/4	f	f		3					
3.20	3	P-ZF72	P-1/4	f	f		3					
3.20	4	P-ZF73	P-1/4	m	m							2
3.20	5	P-ZF74	P-1/4	f	f		3					
3.20	6	P-ZF75	P-1/4	f	f		3		1		2	
3.20	7	P-ZF76	P-1/4	m	m							2
3.20	8	P-ZF77	P-1/4	m	m							2
3.20	9	P-ZF78	P-1/4	m	m							2
3.20	10	P-ZF79	P-1/4	m	f		2					
10.0	1	P-ZF80	P-2/1	f	f	Hermaphrodite	1					1
10.0	2	P-ZF81	P-2/1	f	f		3					
10.0	3	P-ZF82	P-2/1	m	f		2					

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage
10.0	4	P-ZF83	P-2/1	m	m							2
10.0	5	P-ZF84	P-2/1	f	f		2					
10.0	6	P-ZF85	P-2/1	f	f		2		2	1	1	
10.0	7	P-ZF86	P-2/1	m	m							2
10.0	8	P-ZF87	P-2/1	m	m							3
10.0	9	P-ZF88	P-2/1	m	m							2
10.0	10	P-ZF89	P-2/1	m	m							2
10.0	1	P-ZF90	P-2/2	m	m							1
10.0	2	P-ZF91	P-2/2	m	m							2
10.0	3	P-ZF92	P-2/2	f	f		2					
10.0	4	P-ZF93	P-2/2	f	f		3					
10.0	5	P-ZF94	P-2/2	f	f		3					
10.0	6	P-ZF95	P-2/2	f	f		2					
10.0	7	P-ZF96	P-2/2	m	f		2					
10.0	8	P-ZF97	P-2/2	m	m							2
10.0	9	P-ZF98	P-2/2	m	m							2
10.0	10	P-ZF99	P-2/2	m	m							2

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage
10.0	1	P-ZF100	P-2/3	f	f		3	1		1		
10.0	2	P-ZF101	P-2/3	m	m							1
10.0	3	P-ZF102	P-2/3	f	f		3					
10.0	4	P-ZF103	P-2/3	f	f		3		1	2		
10.0	5	P-ZF104	P-2/3	m	m							2
10.0	6	P-ZF105	P-2/3	f	f		3					
10.0	7	P-ZF106	P-2/3	m	m							2
10.0	8	P-ZF107	P-2/3	f	f		3		1	2	1	
10.0	9	P-ZF108	P-2/3	f	f		3		1		1	
10.0	10	P-ZF109	P-2/3	m	m							2
10.0	1	P-ZF110	P-2/4	m	m							3
10.0	2	P-ZF111	P-2/4	f	f		3					
10.0	3	P-ZF112	P-2/4	m	m							2
10.0	4	P-ZF113	P-2/4	m	m							2
10.0	5	P-ZF114	P-2/4	m	m							2
10.0	6	P-ZF115	P-2/4	m	m							2
10.0	7	P-ZF116	P-2/4	f	f		3	1			2	

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage
10.0	8	P-ZF117	P-2/4	f	f		3		1	2		
10.0	9	P-ZF118	P-2/4	f	f		3				1	
10.0	10	P-ZF119	P-2/4	f	f		3					
32.0	1	P-ZF120	P-3/1	f	f		3					
32.0	2	P-ZF121	P-3/1	f	f		3					
32.0	3	P-ZF122	P-3/1	f	f		4					
32.0	4	P-ZF123	P-3/1	f	f		2		1	1		
32.0	5	P-ZF124	P-3/1	m	m							2
32.0	6	P-ZF125	P-3/1	m	m							2
32.0	7	P-ZF126	P-3/1	f	f		3					
32.0	8	P-ZF127	P-3/1	m	m							2
32.0	9	P-ZF128	P-3/1	m	m							2
32.0	10	P-ZF129	P-3/1	m	m							2
32.0	1	P-ZF130	P-3/2	m	m							3
32.0	2	P-ZF131	P-3/2	f	f		3					
32.0	3	P-ZF132	P-3/2	m	m							2
32.0	4	P-ZF133	P-3/2	f	f		3		2	1	1	

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage
32.0	5	P-ZF134	P-3/2	m	m							2
32.0	6	P-ZF135	P-3/2	f	f		3	2			1	
32.0	7	P-ZF136	P-3/2	f	f		3					
32.0	8	P-ZF137	P-3/2	m	m							2
32.0	9	P-ZF138	P-3/2	m	m							3
32.0	10	P-ZF139	P-3/2	f	f		3	1			2	
32.0	1	P-ZF140	P-3/3	f	f		3					
32.0	2	P-ZF141	P-3/3	f	f		2					
32.0	3	P-ZF142	P-3/3	f	f		3		1			
32.0	4	P-ZF143	P-3/3	m	m							2
32.0	5	P-ZF144	P-3/3	f	f		3		1	2		
32.0	6	P-ZF145	P-3/3	m	m							3
32.0	7	P-ZF146	P-3/3	m	m							2
32.0	8	P-ZF147	P-3/3	f	f		3					
32.0	9	P-ZF148	P-3/3	m	m							2
32.0	10	P-ZF149	P-3/3	m	m							2
32.0	1	P-ZF150	P-3/4	f	f		3					

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage
32.0	2	P-ZF151	P-3/4	m	m							3
32.0	3	P-ZF152	P-3/4	f	f		2					
32.0	4	P-ZF153	P-3/4	f	f		3					
32.0	5	P-ZF154	P-3/4	m	m							3
32.0	6	P-ZF155	P-3/4	f	f		3	1		3		
32.0	7	P-ZF156	P-3/4	m	m							2
32.0	8	P-ZF157	P-3/4	m	m							2
32.0	9	P-ZF158	P-3/4	m	m							3
100	1	P-ZF159	P-4/1	m	m							2
100	2	P-ZF160	P-4/1	f	f		3					
100	3	P-ZF161	P-4/1	f	f		3					
100	4	P-ZF162	P-4/1	f	f		4					
100	5	P-ZF163	P-4/1	f	f		3					
100	6	P-ZF164	P-4/1	m	m							2
100	7	P-ZF165	P-4/1	m	m							3
100	8	P-ZF166	P-4/1	f	f		3					
100	9	P-ZF167	P-4/1	m	m							2

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage
100	10	P-ZF168	P-4/1	m	m							2
100	1	P-ZF169	P-4/2	f	f		3		1			
100	2	P-ZF170	P-4/2	f	f		3	2			1	
100	3	P-ZF171	P-4/2	f	f		3					
100	4	P-ZF172	P-4/2	f	f		3					
100	5	P-ZF173	P-4/2	f	f		3		2	1		
100	6	P-ZF174	P-4/2	m	m							3
100	7	P-ZF175	P-4/2	m	m							2
100	8	P-ZF176	P-4/2	m	m							2
100	9	P-ZF177	P-4/2	m	m							3
100	10	P-ZF178	P-4/2	m	m							2
100	1	P-ZF179	P-4/3	f	f		3					
100	2	P-ZF180	P-4/3	f	f		2					
100	3	P-ZF181	P-4/3	m	m							2
100	4	P-ZF182	P-4/3	f	f		3					
100	5	P-ZF183	P-4/3	f	f		2					
100	6	P-ZF184	P-4/3	m	m							2

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage
100	7	P-ZF185	P-4/3	f	f		3					
100	8	P-ZF186	P-4/3	m	m							2
100	9	P-ZF187	P-4/3	m	m							2
100	10	P-ZF188	P-4/3	m	m							2
100	1	P-ZF189	P-4/4	f	f		3					
100	2	P-ZF190	P-4/4	m	m							2
100	3	P-ZF191	P-4/4	m	m							2
100	4	P-ZF192	P-4/4	f	f		3					
100	5	P-ZF193	P-4/4	m	m							2
100	6	P-ZF194	P-4/4	f	f		2	1			2	
100	7	P-ZF195	P-4/4	f	f		3					
100	8	P-ZF196	P-4/4	m	m							2
100	9	P-ZF197	P-4/4	m	m							2
100	10	P-ZF198	P-4/4	m	m							2
320	1	P-ZF199	P-5/1	f	f		3				3	
320	2	P-ZF200	P-5/1	f	f		3					
320	3	P-ZF201	P-5/1	f	f		3					

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage
320	4	P-ZF202	P-5/1	f	f		3					
320	5	P-ZF203	P-5/1	m	m							2
320	6	P-ZF204	P-5/1	f	f		2				1	
320	7	P-ZF205	P-5/1	m	m							2
320	8	P-ZF206	P-5/1	m	m							1
320	9	P-ZF207	P-5/1	m	m							2
320	10	P-ZF208	P-5/1	m	m							2
320	1	P-ZF209	P-5/2	f	f		1			4		
320	2	P-ZF210	P-5/2	f	f		3			1		
320	3	P-ZF211	P-5/2	f	f		3				2	
320	4	P-ZF212	P-5/2	m	m							2
320	5	P-ZF213	P-5/2	f	f		3					
320	6	P-ZF214	P-5/2	m	m							2
320	7	P-ZF215	P-5/2	m	m							2
320	8	P-ZF216	P-5/2	m	m							2
320	9	P-ZF217	P-5/2	m	m							2
320	10	P-ZF218	P-5/2	m	m							2

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage
320	1	P-ZF219	P-5/3	m	m							2
320	2	P-ZF220	P-5/3	f	f		3					
320	3	P-ZF221	P-5/3	m	m							2
320	4	P-ZF222	P-5/3	f	f		3				1	
320	5	P-ZF223	P-5/3	f	f		3		2			
320	6	P-ZF224	P-5/3	m	m							3
320	7	P-ZF225	P-5/3	f	f		2			1	1	
320	8	P-ZF226	P-5/3	m	m							2
320	9	P-ZF227	P-5/3	f	f		3					
320	10	P-ZF228	P-5/3	m	m							2
320	1	P-ZF229	P-5/4	m	m							2
320	2	P-ZF230	P-5/4	f	f		3				2	
320	3	P-ZF231	P-5/4	f	f		2			1		
320	4	P-ZF232	P-5/4	f	f		3					
320	5	P-ZF233	P-5/4	f	f		2					
320	6	P-ZF234	P-5/4	m	m							2
320	7	P-ZF235	P-5/4	f	f		2				2	

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage
320	8	P-ZF236	P-5/4	m	m							2
320	9	P-ZF237	P-5/4	m	m							2
320	10	P-ZF238	P-5/4	m	m							2

m = male; f = female

Severity grades: 0 = not observable, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe

Table 82: F_0 generation, maturation stage males; control, 3.20 and 10.0 ng Dienogest/L (nominal), respective 3.51 and 10.3 ng Dienogest/L (mean measured); (based on histological data provided in C.1.13)

	Nominal concentration Dienogest [ng/L]											
	Control				**3.20**				**10.0**			
	Mean measured concentration Dienogest [ng/L]											
	Control				**3.51**				**10.3**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Maturation stage males**											
1	-	2	-	1	-	2	-	-	1(*)	1	-	3
2	2	-	2	-	-	-	2	-	-	2	1	-
3	-	2	-	2	-	-	-	-	-	-	-	2
4	2	2	-	2	2	2	-	2	2	-	-	2
5	2	-	1	-	3	-	-	-	-	-	2	2
6	-	-	-	-	2	2	2	-	-	-	-	2
7	2	2	2	-	2	-	-	2	2	-	2	-
8	-	-	-	-	-	-	2	2	3	2	-	-
9	2	2	2	2	2	2	2	2	2	2	-	-
10	-	-	-	3	3	-	3	-	2	2	2	-
Median	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**

(*) The fish was diagnosed as a hermaphrodite, and thus not included in calculation of the median.

Table 83: F_0 generation, maturation stage males; 32, 100 and 320 ng Dienogest/L (nominal), respective 31.7, 105 and 335 ng Dienogest/L (mean measured); (based on histological data provided in C.1.13)

	Nominal concentration Dienogest [ng/L]											
	32.0				100				320			
	Mean measured concentration Dienogest [ng/L]											
	31.7				105				335			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Maturation stage males**											
1	-	3	-	-	2	-	-	-	-	-	2	2
2	-	-	-	3	-	-	-	2	-	-	-	-
3	-	2	-	-	-	-	2	2	-	-	2	-
4	-	-	2	-	-	-	-	-	-	2		-
5	2	2	-	3	-	-	-	2	2	-	-	-
6	2	-	3	-	2	3	2	-	-	2	3	2
7	-	-	2	2	3	2	-	-	2	2	-	-
8	2	2	-	2	-	2	2	2	1	2	2	2
9	2	3	2	3	2	3	2	2	2	2	-	2
10	2	-	2	-	2	2	2	2	2	2	2	2
Median	**2.0**	**2.0**	**2.0**	**3.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**

Table 84: F_0 generation, maturation stage females; control, 3.20 and 10.0 ng Dienogest/L(nominal), respective 3.51 and 10.3 ng Dienogest/L (mean measured); (based on histological data provided in C.1.13)

	Nominal concentration Dienogest [ng/L]											
	Control				3.20				10.0			
	Mean measured concentration Dienogest [ng/L]											
	Control				3.51				10.3			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Maturation stage females											
1	3	-	4	-	3	-	3	3	1(*)	-	3	-
2	-	3	-	3	2	2	-	3	3	-	-	3
3	3	-	2	-	3	3	3	3	2	2	3	-
4	-	-	3	-	-	-	3	-	-	3	3	-
5	-	3	-	4	-	2	2	3	2	3	-	-
6	3	2	3	3	-	-	-	3	2	2	3	-
7	-	-	-	2	-	2	2	-	-	2	-	3
8	3	3	2	3	2	2	-	-	-	-	3	3
9	-	-	-	-	-	-	-	-	-	-	3	3
10	3	2	-	-	-	1	-	2	-	-	-	3
Median	**3.0**	**3.0**	**3.0**	**3.0**	**2.5**	**2.0**	**3.0**	**3.0**	**2.0**	**2.0**	**3.0**	**3.0**

(*) The fish was diagnosed as a hermaphrodite, thus not included in calculation of the median.

Table 85: **F_0 generation, maturation stage females; 32.0, 100 and 320 ng Dienogest/L (nominal), respective 31.7, 105 and 335 ng Dienogest/L (mean measured); (based on histological data provided in C.1.13)**

	Nominal concentration Dienogest [ng/L]											
	32.0				100				320			
	Mean measured concentration Dienogest [ng/L]											
	31.7				105				335			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Maturation stage females											
1	3	-	3	3		3	3	3	3	1	-	-
2	3	3	2	-	3	3	2	-	3	3	3	3
3	4	-	3	2	3	3	-	-	3	3	-	2
4	2	3	-	3	4	3	3	3	3	-	3	3
5	-	-	3	-	3	3	2	-	-	3	3	2
6	-	3	-	3	-	-	-	2	2	-	-	-
7	3	3	-	-	-	-	3	3	-	-	2	2
8	-	-	3	-	3	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	3	-
10	-	3	-	-	-	-	-	-	-	-	-	-
Median	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	**2.0**

Table 86: **F_0 generation, maturation stage, summary**

		Nominal concentration Dienogest [ng/L]					
		Control	3.20	10.0	32.0	100	320
		Mean measured concentration Dienogest [ng/L]					
	Replicate	Control	3.51	10.3	31.7	105	335
Maturation stage males, median value	A	2	2	2	2	2	2
	B	2	2	2	2	2	2
	C	2	2	2	2	2	2
	D	2	2	2	3	2	2
Maturation stage females, median value	A	3	3	2	3	3	3
	B	3	2	2	3	3	3
	C	3	3	3	3	3	3
	D	3	3	3	3	3	2

Table 87: F_0, increased oocyte atresia, females; control, 3.20 and 10.0 ng Dienogest/L(nominal), respective 3.51 and 10.3 ng Dienogest/L (mean measured); (based on histological data provided in C.1.13)

	Nominal concentration Dienogest [ng/L]											
	Control				3.20				10.0			
	Mean measured concentration Dienogest [ng/L]											
	Control				3.51				10.3			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Increased oocyte atresia, females											
1	1	-	1	-	2	-	0	1	0(*)	-	1	-
2	-	1	-	0	0	1	-	0	0	-	-	0
3	0	-	0	-	0	0	0	0	0	0	0	-
4	-	-	0	-	-	-	0	-	-	0	0	-
5	-	0	-	0	-	1	0	0	0	0	-	-
6	0	2	0	0	-	-	-	0	0	0	0	-
7	-	-	-	0	-	0	0	-	-	0	-	1
8	0	0	0	0	0	0	-	-	-	-	0	0
9	-	-	-	-	-	-	-	-	-	-	0	0
10	1	0	-	-	-	0	-	0	-	-	-	0
Median	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

(*) The fish was diagnosed as a hermaphrodite, thus was not included in calculation of the median.

Table 88: **F_0, increased oocyte atresia, females 32.0, 100 and 320 ng Dienogest/L (nominal), respective 31.7, 105 and 335 ng Dienogest/L (mean measured); (based on histological data provided in C.1.13)**

	Nominal concentration Dienogest [ng/L]											
	32.0				100				320			
	Mean measured concentration Dienogest [ng/L]											
	31.7				105				335			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Increased oocyte atresia, females											
1	0	-	0	0	-	0	0	0	0	0	-	-
2	0	0	0	-	0	2	0	-	0	0	0	0
3	0	-	0	0	0	0	-	-	0	0	-	0
4	0	0	-	0	0	0	0	0	0	-	0	0
5	-	-	0	-	0	0	0	-	-	0	0	0
6	-	2	-	1	-	-	-	1	0	-	-	-
7	0	0	-	-	-	-	0	0	-	-	0	0
8	-	-	0	-	0	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	0	-
10	-	1	-	-	-	-	-	-	-	-	-	-
Median	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

**Table 89: F_0, egg debris, females
control, 3.20 and 10.0 ng Dienogest/L (nominal), respective 3.51 and 10.3 ng Dienogest/L (mean measured); (based on histological data provided in C.1.13)**

	Nominal concentration Dienogest [ng/L]											
	control				3.20				10.0			
	Mean measured concentration Dienogest [ng/L]											
	Control				3.51				10.3			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Egg debris, females**											
1	0	-	0	-	2	-	0	2	0(*)	-	0	-
2	-	1	-	0	0	0	-	0	0	-	-	0
3	0	-	0	-	2	0	0	0	0	0	0	-
4	-	-	0	-	-	-	0	-	-	0	1	-
5	-	1	-	0	-	2	2	0	0	0	-	-
6	0	0	0	0	-	-	-	1	2	0	0	-
7	-	-	-	0	-	2	1	-	-	0	-	0
8	0	0	0	0	0	0	-	-	-	-	1	1
9	-	-	-	-	-	-	-	-	-	-	1	0
10	0	0	-	-	-	0	-	0	-	-	-	0
Median	**0.0**	**0.0**	**0.0**	**0.0**	**1.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.5**	**0.0**

(*) The fish was diagnosed as a hermaphrodite, and thus not included in calculation of the median.

Table 90: F_0, egg debris, females 32.0, 100 and 320 ng Dienogest/L (nominal), respective 31.7, 105 and 335 ng Dienogest/L (mean measured); (based on histological data provided in C.1.13)

	Nominal concentration Dienogest [ng/L]											
	32.0				100				320			
	Mean measured concentration Dienogest [ng/L]											
	31.7				105				335			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Egg debris, females											
1	0	-	0	0	-	1	0	0	0	0	-	-
2	0	0	0	-	0	0	0	-	0	0	0	0
3	0	-	1	0	0	0	-	-	0	0	-	0
4	1	2	-	0	0	0	0	0	0	-	0	0
5	-	-	1	-	0	2	0	-	-	0	2	0
6	-	0	-	0	-	-	-	0	0	-	-	-
7	0	0	-	-	-	-	0	0	-	-	0	0
8	-	-	0	-	0	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	0	-
10	-	0	-	-	-	-	-	-	-	-	-	-
Median	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Table 91: F_0, granulomatous inflammation, females, control, 3.20 and 10.0 ng Dienogest/L (nominal), respective 3.51 and 10.3 ng Dienogest/L (mean measured); (based on histological data provided in C.1.13)

	Nominal concentration Dienogest [ng/L]											
	Control				3.20				10.0			
	Mean measured concentration Dienogest [ng/L]											
	Control				3.51				10.3			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Granulomatous inflammation, females											
1	0	-	2	-	0	-	0	1	0(*)	-	1	-
2	-	1	-	0	0	0	-	0	0	-	-	0
3	0	-	0	-	0	0	0	0	0	0	0	-
4	-	-	0	-	-	-	0	-	-	0	2	-
5	-	0	-	0	-	0	0	0	0	0	-	-
6	0	0	0	1	-	-	-	0	1	0	0	-
7	-	-	-	0	-	0	0	-	-	0	-	0
8	0	0	0	0	0	0	-	-	-	-	2	2
9	-	-	-	-	-	-	-	-	-	-	0	0
10	0	0	-	-	-	0	-	0	-	-	-	0
Median	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.5**	**0.0**

(*) The fish was diagnosed as a hermaphrodite, and thus not included in calculation of the median.

Table 92: F_0, granulomatous inflammation, females, 32.0, 100 and 320 ng Dienogest/L (nominal), respective 31.7, 105 and 335 ng Dienogest/L (mean measured); (based on histological data provided in C.1.13)

	Nominal concentration Dienogest [ng/L]											
	32.0				100				320			
	Mean measured concentration Dienogest [ng/L]											
	31.7				105				335			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Granulomatous inflammation, females											
1	0	-	0	0	-	0	0	0	0	4	-	-
2	0	0	0	-	0	0	0	-	0	1	0	0
3	0	-	0	0	0	0	-	-	0	0	-	1
4	1	1	-	0	0	0	0	0	0	-	0	0
5	-	-	2	-	0	1	0	-	-	0	0	0
6	-	0	-	3	-	-	-	0	0	-	-	-
7	0	0	-	-	-	-	0	0	-	-	1	0
8	-	-	0	-	0	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	0	-
10	-	0	-	-	-	-	-	-	-	-	-	-
Median	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.5**	**0.0**	**0.0**

Table 93: F_0, increased post-ovulatory follicles, females, control, 3.20 and 10.0 ng Dienogest/L (nominal), respective 3.51 and 10.3 ng Dienogest/L (mean measured); (based on histological data provided in C.1.13)

	Nominal concentration Dienogest [ng/L]											
	Control				3.20				10.0			
	Mean measured concentration Dienogest [ng/L]											
	Control				3.51				10.3			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Increased post-ovulatory follicles, females											
1	0	-	0	-	0	-	0	0	0(*)	-	0	-
2	-	0	-	1	0	0	-	0	0	-	-	0
3	0	-	0	-	1	0	0	0	0	0	0	-
4	-	-	0	-	-	-	0	-	-	0	0	-
5	-	2	-	0	-	0	1	0	0	0	-	-
6	0	0	0	0	-	-	-	2	1	0	0	-
7	-	-	-	0	-	0	1	-	-	0	-	2
8	0	0	2	0	1	0	-	-	-	-	1	0
9	-	-	-	-	-	-	-	-	-	-	1	1
10	1	1	-	-	-	0	-	0	-	-	-	0
Median	**0.0**	**0.0**	**0.0**	**0.0**	**0.5**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

(*) The fish was diagnosed as a hermaphrodite, and thus not included in calculation of the median.

Table 94: F_0, increased post-ovulatory follicles, females 32.0, 100 and 320 ng Dienogest/L (nominal), respective 31.7, 105 and 335 ng Dienogest/L (mean measured); (based on histological data provided in C.1.13)

	Nominal concentration Dienogest [ng/L]											
	32.0				100				320			
	Mean measured concentration Dienogest [ng/L]											
	31.7				105				335			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Increased post-ovulatory follicles, females**											
1	0	-	0	0	-	0	0	0	3	0	-	-
2	0	0	0	-	0	1	0	-	0	0	0	2
3	0	-	0	0	0	0	-	-	0	2	-	0
4	0	1	-	0	0	0	0	0	0	-	1	0
5	-	-	0	-	0	0	0	-	-	0	0	0
6	-	1	-	0	-	-	-	2	1	-	-	-
7	0	0	-	-	-	-	0	0	-	-	1	2
8	-	-	0	-	0	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	0	-
10	-	2	-	-	-	-	-	-	-	-	-	-
Median	**0.0**	**1.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Table 95: F_0 generation, grading of lesions severity, summary

		Nominal concentration Dienogest [ng/L]					
		Control	3.20	10.0	32.0	100	320
		Mean measured concentration Dienogest [ng/L]					
	Replicate	Control	3.51	10.3	31.7	105	335
Increased oocyte atresia, females, median value	A	0.0	0.0	0.0	0.0	0.0	0.0
	B	0.0	0.0	0.0	0.0	0.0	0.0
	C	0.0	0.0	0.0	0.0	0.0	0.0
	D	0.0	0.0	0.0	0.0	0.0	0.0
Egg debris, females, median value	A	0.0	1.0	0.0	0.0	0.0	0.0
	B	0.0	0.0	0.0	0.0	0.0	0.0
	C	0.0	0.0	0.5	0.0	0.0	0.0
	D	0.0	0.0	0.0	0.0	0.0	0.0
Granulomatous inflammation, females, median value	A	0.0	0.0	0.0	0.0	0.0	0.0
	B	0.0	0.0	0.0	0.0	0.0	0.5
	C	0.0	0.0	0.5	0.0	0.0	0.0
	D	0.0	0.0	0.0	0.0	0.0	0.0
Increased post-ovulatory follicles, females, median value	A	0.0	0.5	0.0	0.0	0.0	0.0
	B	0.0	0.0	0.0	1.0	0.0	0.0
	C	0.0	0.0	0.0	0.0	0.0	0.0
	D	0.0	0.0	0.0	0.0	0.0	0.0

C.1.14 Histopathology of fish gonads, F_1 generation

Table 96: Histology raw data, F_1 generation

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
control	1	F1-ZF1	0/1 1	m	m							2			
control	2	F1-ZF2	0/1 2	m	m							2			
control	3	F1-ZF3	0/1 3	m	m							2			
control	4	F1-ZF4	0/1 4	m	m							2	1		
control	5	F1-ZF5	0/1 5	f	f		2				1				
control	6	F1-ZF6	0/1 6	m	m							2			
control	7	F1-ZF7	0/1 7	m	m							2			
control	8	F1-ZF8	0/1 8	f	f		3								
control	9	F1-ZF9	0/1 9	f	f		2								
control	10	F1-ZF10	0/1 10	m	m							3			
control	11	F1-ZF11	0/1 11	m	m							2	1		
control	12	F1-ZF12	0/1 12	m	m							2			
control	13	F1-ZF13	0/1 13	f	f		3								

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
control	14	F1-ZF14	0/1 14	m	m							2			
control	15	F1-ZF15	0/1 15	f	f		2								
control	16	F1-ZF16	0/1 16	f	f		2								
control	17	F1-ZF17	0/1 17	f	f		2								
control	18	F1-ZF18	0/1 18	m	m							2			
control	19	F1-ZF19	0/1 19	m	m							2			
control	20	F1-ZF20	0/1 20	m	m							2			
control	1	F1-ZF21	0/2 1	f	f		3								
control	2	F1-ZF22	0/2 2	f	f		3								
control	3	F1-ZF23	0/2 3	f	f		3	1							
control	4	F1-ZF24	0/2 4	f	f		3								
control	5	F1-ZF25	0/2 5	f	f		4								
control	6	F1-ZF26	0/2 6	m	m							1			
control	7	F1-ZF27	0/2 7	m	m							2			
control	8	F1-ZF28	0/2 8	m	m							2			
control	9	F1-ZF29	0/2 9	m	m							3	1		

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
control	10	F1-ZF30	0/2 10	m	m							2			
control	11	F1-ZF31	0/2 11	f	f		3								
control	12	F1-ZF32	0/2 12	m	m							2			
control	13	F1-ZF33	0/2 13	f	f		2		1						
control	14	F1-ZF34	0/2 14	f	f		2				1				
control	15	F1-ZF35	0/2 15	f	f		3								
control	16	F1-ZF36	0/2 16	f	f		3								
control	17	F1-ZF37	0/2 17	m	m							2			
control	18	F1-ZF38	0/2 18	m	m							2			
control	19	F1-ZF39	0/2 19	f	f		2								
control	20	F1-ZF40	0/2 20	m	m							2			
control	1	F1-ZF41	0/3 1	f	f		2		1	1	1				
control	2	F1-ZF42	0/3 2	f	f		2								
control	3	F1-ZF43	0/3 3	f	f		3		1						
control	4	F1-ZF44	0/3 4	m	m							2			
control	5	F1-ZF45	0/3 5	m	m							2			

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
control	6	F1-ZF46	0/3 6	m	m							1			
control	7	F1-ZF47	0/3 7	m	m							2			
control	8	F1-ZF48	0/3 8	m	m							2			
control	9	F1-ZF49	0/3 9	m	m							2			
control	10	F1-ZF50	0/3 10	m	m							2			
control	11	F1-ZF51	0/3 11	m	m							3			
control	12	F1-ZF52	0/3 12	f	f		2								
control	13	F1-ZF53	0/3 13	f	f		3			1					
control	14	F1-ZF54	0/3 14	m	m							2			
control	15	F1-ZF55	0/3 15	f	f		3								
control	16	F1-ZF56	0/3 16	m	m							2			
control	17	F1-ZF57	0/3 17	m	m							2			
control	18	F1-ZF58	0/3 18	m	m							2			
control	19	F1-ZF59	0/3 19	m	m							2			
control	1	F1-ZF60	0/4 1	f	f		3		2						
control	2	F1-ZF61	0/4 2	m	m							2			

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
control	3	F1-ZF62	0/4 3	f	f		3								
control	4	F1-ZF63	0/4 4	m	f		3								
control	5	F1-ZF64	0/4 5	m	m							3	1		
control	6	F1-ZF65	0/4 6	m	m							2			
control	7	F1-ZF66	0/4 7	m	m							3	2		
control	8	F1-ZF67	0/4 8	m	m							2	1		
control	9	F1-ZF68	0/4 9	f	f		3		1	2					
control	10	F1-ZF69	0/4 10	f	f		3								
control	11	F1-ZF70	0/4 11	f	f		2								
control	12	F1-ZF71	0/4 12	m	m							2			
control	13	F1-ZF72	0/4 13	m	m							3			
control	14	F1-ZF73	0/4 14	f	f		2								
control	15	F1-ZF74	0/4 15	f	m							2			
control	16	F1-ZF75	0/4 16	m	m							2			
control	17	F1-ZF76	0/4 17	m	m							2			
control	18	F1-ZF77	0/4 18	m	m							2			

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
control	19	F1-ZF78	0/4 19	m	m							2			
control	20	F1-ZF79	0/4 20	m	m							3			
3.20	1	F1-ZF80	1/1 1	m	m							2			
3.20	2	F1-ZF81	1/1 2	m	m							3			
3.20	3	F1-ZF82	1/1 3	f	f		3								
3.20	4	F1-ZF83	1/1 4	f	f		2								
3.20	5	F1-ZF84	1/1 5	m	m							2			
3.20	6	F1-ZF85	1/1 6	f	f		2		1						
3.20	7	F1-ZF86	1/1 7	f	f		4								
3.20	8	F1-ZF87	1/1 8	m	m							2			
3.20	9	F1-ZF88	1/1 9	f	f		3								
3.20	10	F1-ZF89	1/1 10	m	m							2			
3.20	11	F1-ZF90	1/1 11	f	f		3								
3.20	12	F1-ZF91	1/1 12	m	m							2			
3.20	13	F1-ZF92	1/1 13	m	m							2			
3.20	14	F1-ZF93	1/1 14	f	f		3								

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
3.20	15	F1-ZF94	1/1 15	m	m							2			
3.20	16	F1-ZF95	1/1 16	m	m							2			
3.20	17	F1-ZF96	1/1 17	m	m							2			
3.20	18	F1-ZF97	1/1 18	f	f		3			1					
3.20	19	F1-ZF98	1/1 19	f	f		3		2	1					
3.20	20	F1-ZF99	1/1 20	m	m							3			
3.20	1	F1-ZF100	1/2 1	m	m							3			
3.20	2	F1-ZF101	1/2 2	f	f		3								
3.20	3	F1-ZF102	1/2 3	f	f		2								
3.20	4	F1-ZF103	1/2 4	f	f		3								
3.20	5	F1-ZF104	1/2 5	m	m							2			
3.20	6	F1-ZF105	1/2 6	f	f		2				1				
3.20	7	F1-ZF106	1/2 7	f	f		3								
3.20	8	F1-ZF107	1/2 8	f	f		3								
3.20	9	F1-ZF108	1/2 9	f	f		3								
3.20	10	F1-ZF109	1/2 10	f	f		3								

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
3.20	11	F1-ZF110	1/2 11	f	f		3								
3.20	12	F1-ZF111	1/2 12	f	f		3								
3.20	13	F1-ZF112	1/2 13	m	m							2			
3.20	14	F1-ZF113	1/2 14	m	m							2			
3.20	15	F1-ZF114	1/2 15	m	m							2			
3.20	16	F1-ZF115	1/2 16	m	m							2			
3.20	17	F1-ZF116	1/2 17	m	m							2	1		
3.20	18	F1-ZF117	1/2 18	m	m							2			
3.20	19	F1-ZF118	1/2 19	m	m							2			
3.20	1	F1-ZF119	1/3 1	f	f		3								
3.20	2	F1-ZF120	1/3 2	m	m							3	1		
3.20	3	F1-ZF121	1/3 3	m	m							2			
3.20	4	F1-ZF122	1/3 4	m	m							3			
3.20	5	F1-ZF123	1/3 5	m	m							2			
3.20	6	F1-ZF124	1/3 6	m	m							3			
3.20	7	F1-ZF125	1/3 7	m	m							2			

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
3.20	8	F1-ZF126	1/3 8	f	f		2								
3.20	9	F1-ZF127	1/3 9	f	f		3								
3.20	10	F1-ZF128	1/3 10	f	f		3								
3.20	11	F1-ZF129	1/3 11	m	m							2			
3.20	12	F1-ZF130	1/3 12	m	m							2			
3.20	13	F1-ZF131	1/3 13	m	m							2			
3.20	14	F1-ZF132	1/3 14	f	f		3				1				
3.20	15	F1-ZF133	1/3 15	f	f		2								
3.20	16	F1-ZF134	1/3 16	m	m							2			
3.20	17	F1-ZF135	1/3 17	m	m							3			
3.20	18	F1-ZF136	1/3 18	m	m							2			
3.20	19	F1-ZF137	1/3 19	m	m							3			
3.20	20	F1-ZF138	1/3 20	m	m							2			
3.20	1	F1-ZF139	1/4 1	m	m							3	1		
3.20	2	F1-ZF140	1/4 2	m	m							2			
3.20	3	F1-ZF141	1/4 3	m	m							2			

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
3.20	4	F1-ZF142	1/4 4	m	m							2			
3.20	5	F1-ZF143	1/4 5	m	m							2			
3.20	6	F1-ZF144	1/4 6	f	f		3		2	1	1				
3.20	7	F1-ZF145	1/4 7	f	f		2								
3.20	8	F1-ZF146	1/4 8	m	m							2			
3.20	9	F1-ZF147	1/4 9	f	f		3		1						
3.20	10	F1-ZF148	1/4 10	f	f		2								
3.20	11	F1-ZF149	1/4 11	f	f		3								
3.20	12	F1-ZF150	1/4 12	f	f		3								
3.20	13	F1-ZF151	1/4 13	m	m							2			
3.20	14	F1-ZF152	1/4 14	f	f		3								
3.20	15	F1-ZF153	1/4 15	f	f		2		1						
3.20	16	F1-ZF154	1/4 16	m	m							2			
3.20	17	F1-ZF155	1/4 17	f	f		3				1				
3.20	18	F1-ZF156	1/4 18	m	m							2			
3.20	19	F1-ZF157	1/4 19	m	m							2			

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
3.20	20	F1-ZF158	1/4 20	m	m							3			
10.0	1	F1-ZF159	2/1 1	f	f		2			1					
10.0	2	F1-ZF160	2/1 2	f	f		2			1					
10.0	3	F1-ZF161	2/1 3	f	f		2		1						
10.0	4	F1-ZF162	2/1 4	f	f		3								
10.0	5	F1-ZF163	2/1 5	f	f		3								
10.0	6	F1-ZF164	2/1 6	m	m							2			1
10.0	7	F1-ZF165	2/1 7	m	m							2			
10.0	8	F1-ZF166	2/1 8	f	f		3								
10.0	9	F1-ZF167	2/1 9	f	f		4								
10.0	10	F1-ZF168	2/1 10	f	f		3								
10.0	11	F1-ZF169	2/1 11	f	f		2								
10.0	12	F1-ZF170	2/1 12	m	m							2			
10.0	13	F1-ZF171	2/1 13	f	f		3								
10.0	14	F1-ZF172	2/1 14	f	f		3								
10.0	15	F1-ZF173	2/1 15	m	m							2			

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
10.0	16	F1-ZF174	2/1 16	m	m							2			
10.0	17	F1-ZF175	2/1 17	f	f		3								
10.0	18	F1-ZF176	2/1 18	f	f		3								
10.0	19	F1-ZF177	2/1 19	f	f		2								
10.0	20	F1-ZF178	2/1 20	m	m							1			
10.0	1	F1-ZF179	2/2 1	f	f		3				1				
10.0	2	F1-ZF180	2/2 2	m	m							3			
10.0	3	F1-ZF181	2/2 3	m	m							2			
10.0	4	F1-ZF182	2/2 4	f	f		3				2				
10.0	5	F1-ZF183	2/2 5	m	m							3			
10.0	6	F1-ZF184	2/2 6	m	m							2			
10.0	7	F1-ZF185	2/2 7	f	f		3								
10.0	8	F1-ZF186	2/2 8	f	f		3								
10.0	9	F1-ZF187	2/2 9	f	f		3			1					
10.0	10	F1-ZF188	2/2 10	f	f		3								
10.0	11	F1-ZF189	2/2 11	m	m							2			

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
10.0	12	F1-ZF190	2/2 12	m	m							3			
10.0	13	F1-ZF191	2/2 13	f	f		3				2				
10.0	14	F1-ZF192	2/2 14	f	f		3								
10.0	15	F1-ZF193	2/2 15	m	m							2			
10.0	16	F1-ZF194	2/2 16	f	f		2								
10.0	17	F1-ZF195	2/2 17	m	m							2			
10.0	18	F1-ZF196	2/2 18	m	m							2			
10.0	19	F1-ZF197	2/2 19	f	f		3								
10.0	20	F1-ZF198	2/2 20	m	f		3								
10.0	1	F1-ZF199	2/3 1	f	f		2								
10.0	2	F1-ZF200	2/3 2	m	m							2			
10.0	3	F1-ZF201	2/3 3	f	f		3								
10.0	4	F1-ZF202	2/3 4	m	m							2			
10.0	5	F1-ZF203	2/3 5	m	m							1	1		
10.0	6	F1-ZF204	2/3 6	m	m							2			
10.0	7	F1-ZF205	2/3 7	m	m							2			

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
10.0	8	F1-ZF206	2/3 8	m	m							2			
10.0	9	F1-ZF207	2/3 9	f	f		3								
10.0	10	F1-ZF208	2/3 10	f	f		2				2				
10.0	11	F1-ZF209	2/3 11	f	f		3								
10.0	12	F1-ZF210	2/3 12	f	f		2								
10.0	13	F1-ZF211	2/3 13	m	m							2	1		
10.0	14	F1-ZF212	2/3 14	m	m							3	1		
10.0	15	F1-ZF213	2/3 15	f	f		2								
10.0	16	F1-ZF214	2/3 16	m	m							2			
10.0	17	F1-ZF215	2/3 17	m	m							2			
10.0	18	F1-ZF216	2/3 18	f	f		3		1						
10.0	19	F1-ZF217	2/3 19	m	m							2			
10.0	20	F1-ZF218	2/3 20	m	m							2			
10.0	1	F1-ZF219	2/4 1	f	f		3								
10.0	2	F1-ZF220	2/4 2	f	f		2								
10.0	3	F1-ZF221	2/4 3	f	f		3								

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
10.0	4	F1-ZF222	2/4 4	f	f		2								
10.0	5	F1-ZF223	2/4 5	f	f		2								
10.0	6	F1-ZF224	2/4 6	f	f		3								
10.0	7	F1-ZF225	2/4 7	f	f		3								
10.0	8	F1-ZF226	2/4 8	m	m							2			
10.0	9	F1-ZF227	2/4 9	m	m							2			
10.0	10	F1-ZF228	2/4 10	m	m							2			
10.0	11	F1-ZF229	2/4 11	m	m							2			
10.0	12	F1-ZF230	2/4 12	f	f		3								
10.0	13	F1-ZF231	2/4 13	m	m							2			
10.0	14	F1-ZF232	2/4 14	m	m							3			
10.0	15	F1-ZF233	2/4 15	f	f		2								
10.0	16	F1-ZF234	2/4 16	m	m							2			
10.0	17	F1-ZF235	2/4 17	f	f		2								
10.0	18	F1-ZF236	2/4 18	f	f		3								
10.0	19	F1-ZF237	2/4 19	m	m							2	1		

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
10.0	20	F1-ZF238	2/4 20	f	f		1								
32.0	1	F1-ZF239	3/1 1	m	m							2			
32.0	2	F1-ZF240	3/1 2	f	f		3								
32.0	3	F1-ZF241	3/1 3	m	m							2			
32.0	4	F1-ZF242	3/1 4	f	f		3								
32.0	5	F1-ZF243	3/1 5	m	m							3			
32.0	6	F1-ZF244	3/1 6	f	f		3								
32.0	7	F1-ZF245	3/1 7	m	m							2			
32.0	8	F1-ZF246	3/1 8	m	m							2			
32.0	9	F1-ZF247	3/1 9	f	f		3								
32.0	10	F1-ZF248	3/1 10	m	m							2			
32.0	11	F1-ZF249	3/1 11	f	f		2								
32.0	12	F1-ZF250	3/1 12	m	m							2			
32.0	13	F1-ZF251	3/1 13	m	m							3			
32.0	14	F1-ZF252	3/1 14	m	m							2			
32.0	15	F1-ZF253	3/1 15	f	f		3								

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
32.0	16	F1-ZF254	3/1 16	f	f		3								
32.0	17	F1-ZF255	3/1 17	m	m							2			
32.0	18	F1-ZF256	3/1 18	f	f		2				1				
32.0	19	F1-ZF257	3/1 19	m	m							2			
32.0	20	F1-ZF258	3/1 20	m	m							3			
32.0	1	F1-ZF259	3/2 1	f	f		3				1				
32.0	2	F1-ZF260	3/2 2	f	f		3								
32.0	3	F1-ZF261	3/2 3	f	f		3		1						
32.0	4	F1-ZF262	3/2 4	m	m							2			
32.0	5	F1-ZF263	3/2 5	m	m							3			
32.0	6	F1-ZF264	3/2 6	f	f		3								
32.0	7	F1-ZF265	3/2 7	m	m							2			
32.0	8	F1-ZF266	3/2 8	m	m							2			
32.0	9	F1-ZF267	3/2 9	m	m							2			
32.0	10	F1-ZF268	3/2 10	m	m							3			
32.0	11	F1-ZF269	3/2 11	f	f		3								

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
32.0	12	F1-ZF270	3/2 12	m	m							2			
32.0	13	F1-ZF271	3/2 13	m	m							2			
32.0	14	F1-ZF272	3/2 14	m	m							2			
32.0	15	F1-ZF273	3/2 15	m	m							3			
32.0	16	F1-ZF274	3/2 16	m	m							2			
32.0	1	F1-ZF275	3/3 1	f	f		3								
32.0	2	F1-ZF276	3/3 2	f	f		3								
32.0	3	F1-ZF277	3/3 3	f	f		3								
32.0	4	F1-ZF278	3/3 4	m	m							2			
32.0	5	F1-ZF279	3/3 5	f	f		3		1						
32.0	6	F1-ZF280	3/3 6	f	f		2								
32.0	7	F1-ZF281	3/3 7	m	m							1			
32.0	8	F1-ZF282	3/3 8	m	m							2			
32.0	9	F1-ZF283	3/3 9	m	m							2			
32.0	10	F1-ZF284	3/3 10	f	f		2			1					
32.0	11	F1-ZF285	3/3 11	f	f		3		1						

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
32.0	12	F1-ZF286	3/3 12	f	f		3								
32.0	13	F1-ZF287	3/3 13	f	f		3								
32.0	14	F1-ZF288	3/3 14	m	m							2			
32.0	15	F1-ZF289	3/3 15	f	f		3								
32.0	16	F1-ZF290	3/3 16	f	f		3								
32.0	17	F1-ZF291	3/3 17	f	f		3								
32.0	18	F1-ZF292	3/3 18	m	m							2			
32.0	19	F1-ZF293	3/3 19	m	m							2			
32.0	1	F1-ZF294	3/4 1	f	f		2				2				
32.0	2	F1-ZF295	3/4 2	m	m							2			
32.0	3	F1-ZF296	3/4 3	f	f		3								
32.0	4	F1-ZF297	3/4 4	f	f		3				2				
32.0	5	F1-ZF298	3/4 5	m	m							2			
32.0	6	F1-ZF299	3/4 6	m	m							2			
32.0	7	F1-ZF300	3/4 7	f	f		2								
32.0	8	F1-ZF301	3/4 8	m	m							2			

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
32.0	9	F1-ZF302	3/4 9	f	f		3								
32.0	10	F1-ZF303	3/4 10	f	f		3								
32.0	11	F1-ZF304	3/4 11	f	f		3								
32.0	12	F1-ZF305	3/4 12	f	f		3			1					
32.0	13	F1-ZF306	3/4 13	f	f		3								
32.0	14	F1-ZF307	3/4 14	f	f		3								
32.0	15	F1-ZF308	3/4 15	f	f		3			1					
32.0	16	F1-ZF309	3/4 16	f	f		3								
32.0	17	F1-ZF310	3/4 17	m	m							2			
32.0	18	F1-ZF311	3/4 18	m	m							2			
32.0	19	F1-ZF312	3/4 19	m	m							2			
32.0	20	F1-ZF313	3/4 20	m	m							2	1		
100	1	F1-ZF314	4/2 1	f	f		3								
100	2	F1-ZF315	4/2 2	f	f		3								
100	3	F1-ZF316	4/2 3	f	f		2				1				
100	4	F1-ZF317	4/2 4	f	f		3		2						

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
100	5	F1-ZF318	4/2 5	m	m							3			
100	6	F1-ZF319	4/2 6	m	m							2			
100	7	F1-ZF320	4/2 7	m	m							2			
100	8	F1-ZF321	4/2 8	f	f		3		2						
100	9	F1-ZF322	4/2 9	f	f		3								
100	10	F1-ZF323	4/2 10	f	f		2				1				
100	11	F1-ZF324	4/2 11	f	f		3								
100	12	F1-ZF325	4/2 12	f	f		3								
100	13	F1-ZF326	4/2 13	f	m							2			
100	14	F1-ZF327	4/2 14	m	m							3			
100	15	F1-ZF328	4/2 15	m	m							2			
100	16	F1-ZF329	4/2 16	f	f		3								
100	17	F1-ZF330	4/2 17	f	f		3			1					
100	18	F1-ZF331	4/2 18	m	m							2			
100	19	F1-ZF332	4/2 19	m	m							2			
100	1	F1-ZF333	4/3 1	f	f		3								

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
100	2	F1-ZF334	4/3 2	f	f		3				1				
100	3	F1-ZF335	4/3 3	f	f		3		2	1					
100	4	F1-ZF336	4/3 4	m	m							3	2		2
100	5	F1-ZF337	4/3 5	m	m							3		2	
100	6	F1-ZF338	4/3 6	m	m							2			
100	7	F1-ZF339	4/3 7	m	m							2			
100	1	F1-ZF340	4/4 1	f	f		2				1				
100	2	F1-ZF341	4/4 2	f	f		3								
100	3	F1-ZF342	4/4 3	f	f		3				1				
100	4	F1-ZF343	4/4 4	m	m							2			
100	5	F1-ZF344	4/4 5	f	f		2				1				
100	6	F1-ZF345	4/4 6	m	m							2	1		
100	7	F1-ZF346	4/4 7	f	f		3								
100	8	F1-ZF347	4/4 8	?	f		2								
100	9	F1-ZF348	4/4 9	f	f		3								
100	10	F1-ZF349	4/4 10	f	f		2				2				

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
100	11	F1-ZF350	4/4 11	m	m							2			
100	12	F1-ZF351	4/4 12	m	m							2			
100	13	F1-ZF352	4/4 13	f	f		3								
100	14	F1-ZF353	4/4 14	f	f		3								
100	15	F1-ZF354	4/4 15	f	f		3								
100	16	F1-ZF355	4/4 16	f	f		3								
100	17	F1-ZF356	4/4 17	m	m							2			
320	1	F1-ZF357	5/1 1	f	f		3								
320	2	F1-ZF358	5/1 2	f	f		3								
320	3	F1-ZF359	5/1 3	f	f		3								
320	4	F1-ZF360	5/1 4	m	m							3			
320	5	F1-ZF361	5/1 5	f	f		3								
320	6	F1-ZF362	5/1 6	m	m							2			
320	7	F1-ZF363	5/1 7	m	m							2			
320	8	F1-ZF364	5/1 8	m	m							2			
320	9	F1-ZF365	5/1 9	m	m							2			

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
320	10	F1-ZF366	5/1 10	m	m							2			
320	11	F1-ZF367	5/1 11	m	m							2			
320	1	F1-ZF368	5/2 1	f	f		3								
320	2	F1-ZF369	5/2 2	f	f		3		2	1	1				
320	3	F1-ZF370	5/2 3	f	f		3	1							
320	4	F1-ZF371	5/2 4	f	f		2		1		1				
320	5	F1-ZF372	5/2 5	f	f		2								
320	6	F1-ZF373	5/2 6	f	f		3								
320	7	F1-ZF374	5/2 7	m	m							2			
320	8	F1-ZF375	5/2 8	m	m							1			
320	9	F1-ZF376	5/2 9	m	m							2			
320	1	F1-ZF377	5/3 1	f	f		3								
320	2	F1-ZF378	5/3 2	f	f		2				1				
320	3	F1-ZF379	5/3 3	m	m							3			
320	4	F1-ZF380	5/3 4	f	f		3								
320	5	F1-ZF381	5/3 5	m	m							2			

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
320	6	F1-ZF382	5/3 6	m	m							2			
320	7	F1-ZF383	5/3 7	m	m							2			
320	8	F1-ZF384	5/3 8	m	m							2			
320	9	F1-ZF385	5/3 9	m	m							2			
320	10	F1-ZF386	5/3 10	m	m							2			
320	11	F1-ZF387	5/3 11	m	m							2			
320	12	F1-ZF388	5/3 12	m	m							1			
320	13	F1-ZF389	5/3 13	f	f		4	1	4	3					
320	14	F1-ZF390	5/3 14	f	f		3								
320	15	F1-ZF391	5/3 15	f	f		3	1	2	2	2				
320	16	F1-ZF392	5/3 16	f	f		2								
320	17	F1-ZF393	5/3 17	m	m	Uni-lateral atrophy						2	4		1
320	18	F1-ZF394	5/3 18	f	f		3								
320	19	F1-ZF395	5/3 19	m	m							2			
320	20	F1-ZF396	5/3 20	f	f		2								

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
320	1	F1-ZF397	5/4 1	f	f		3								
320	2	F1-ZF398	5/4 2	m	m							3			
320	3	F1-ZF399	5/4 3	f	f		3								
320	4	F1-ZF400	5/4 4	m	m							1			
320	5	F1-ZF401	5/4 5	f	f		3		1		2				
320	6	F1-ZF402	5/4 6	f	f		2		1		2				
320	7	F1-ZF403	5/4 7	f	f		3								
320	8	F1-ZF404	5/4 8	m	m							2			
320	9	F1-ZF405	5/4 9	m	m							2			
320	10	F1-ZF406	5/4 10	m	m							2			
320	11	F1-ZF407	5/4 11	m	m							3			
320	12	F1-ZF408	5/4 12	f	f		3				1				
320	13	F1-ZF409	5/4 13	f	f		2		1						
320	14	F1-ZF410	5/4 14	m	m							2			
320	15	F1-ZF411	5/4 15	m	m							2			
320	16	F1-ZF412	5/4 16	m	m							2			

Nominal concentration Dienogest [ng/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	Testis-ova	asynchronous germ cell development	granulomatous inflammation
320	17	F1-ZF413	5/4 17	m	m							2			
320	18	F1-ZF414	5/4 18	m	m							3			

m = male; f = female

Severity grades: 0 = not observable, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe

Table 97: F_1 generation, maturation stage males, control, 3.20 and 10.0 ng Dienogest/L(nominal), respective 3.51 and 10.3 ng Dienogest/L (mean measured); (based on histological data provided in C.1.14)

	Nominal concentration Dienogest [ng/L]											
	control				**3.20**				**10.0**			
	Mean measured concentration Dienogest [ng/L]											
	Control				**3.51**				**10.3**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Maturation stage males**											
1	2	-	-	-	2	3	-	3	-	-	-	-
2	2	-	-	2	3	-	3	2	-	3	2	-
3	2	-	-	-	-	-	2	2	-	2	-	-
4	2	-	2	-	-	-	3	2	-	-	2	-
5	-	-	2	3	2	2	2	2	-	3	1	-
6	2	1	1	2	-	-	3	-	2	2	2	-
7	2	2	2	3	-	-	2	-	2	-	2	-
8	-	2	2	2	2	-	-	2	-	-	2	2
9	-	3	2	-	-	-	-	-	-	-	-	2
10	3	2	2	-	2	-	-	-	-	-	-	2
11	2	-	3	-	-	-	2	-	-	2	-	2
12	2	2	-	2	2	-	2	-	2	3	-	-
13	-	-	-	3	2	2	2	2	-	-	2	2
14	2	-	2	-	-	2	-	-	-	-	3	3
15	-	-	-	2	2	2	-	-	2	2	-	-
16	-	-	2	2	2	2	2	2	2	-	2	2
17	-	2	2	2	2	2	3	-	-	2	2	-
18	2	2	2	2	-	2	2	2	-	2	-	-
19	2	-	2	2	-	2	3	2	-	-	2	2
20	2	2	-	3	3	-	2	3	1	-	2	-
Median	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**

Table 98: F_1 generation, maturation stage males, 32.0, 100 and 320 ng Dienogest/L (nominal), respective 31.7, 105 and 335 ng Dienogest/L (mean measured); (based on histological data provided in C.1.14)

	Nominal concentration Dienogest [ng/L]											
	32.0				**100**				**320**			
	Mean measured concentration Dienogest [ng/L]											
	31.7				**105**				**335**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Maturation stage males**											
1	2	-	-	-		-	-	-	-	-	-	-
2	-	-	-	2		-	-	-	-	-	-	3
3	2	-	-	-		-	-	-	-	-	3	-
4	-	2	2	-		-	3	2	3	-	-	1
5	3	3	-	2		3	3	-	-	-	2	-
6	-	-	-	2		2	2	2	2	-	2	-
7	2	2	1	-		2	2	-	2	2	2	-
8	2	2	2	2		-	-	-	2	1	2	2
9	-	2	2	-		-	-	-	2	2	2	2
10	2	3	-	-		-	-	-	2	-	2	2
11	-	-	-	-		-	-	2	2	-	2	3
12	2	2	-	-		-	-	2	-	-	1	-
13	3	2	-	-		2	-	-	-	-	-	-
14	2	2	2	-		3	-	-	-	-	-	2
15	-	3	-	-		2	-	-	-	-	-	2
16	-	2	-	-		-	-	-	-	-	-	2
17	2	-	-	2		-	-	2	-	-	2	2
18	-	-	2	2		2	-	-	-	-	-	3
19	2	-	2	2		2	-	-	-	-	2	-
20	3	-	-	2		-	-	-	-	-	-	-
Median	**2**	**2**	**2**	**2**	**-**	**2**	**3**	**2**	**2**	**2**	**2**	**2**

Table 99: F_1 generation, maturation stage females, control, 3.20 and 10.0 ng Dienogest/L (nominal), respective 3.51 and 10.3 ng Dienogest/L (mean measured); (based on histological data provided in C.1.14)

	Nominal concentration Dienogest [ng/L]											
	control				3.20				10.0			
	Mean measured concentration Dienogest [ng/L]											
	Control				3.51				10.3			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Maturation stage females											
1	-	3	2	3	-	-	3	-	2	3	2	3
2	-	3	2	-	-	3	-	-	2	-	-	2
3	-	3	3	3	3	2	-	-	2	-	3	3
4	-	3	-	3	2	3	-	-	3	3	-	2
5	2	4	-	-	-	-	-	-	3	-	-	2
6	-	-	-	-	2	2	-	3	-	-	-	3
7	-	-	-	-	4	3	-	2	-	3	-	3
8	3	-	-	-	-	3	2	-	3	3	-	-
9	2	-	-	3	3	3	3	3	4	3	3	-
10	-	-	-	3	-	3	3	2	3	3	2	-
11	-	3	-	2	3	3	-	3	2	-	3	-
12	-	-	2	-	-	3	-	3	-	-	2	3
13	3	2	3	-	-	-	-		3	3	-	-
14	-	2	-	2	3	-	3	3	3	3	-	-
15	2	3	3	-	-	-	2	2	-		2	2
16	2	3	-	-	-	-	-	-	-	2	-	-
17	2	-	-	-	-	-	-	3	3	-	-	2
18	-	-	-	-	3	-	-	-	3	-	3	3
19	-	2	-	-	3	-	-	-	2	3	-	-
20	-	-	-	-	-	-	-	-	-	3	-	1
Median	**2**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**

Table 100: F_1 generation, maturation stage females, 32.0, 100 and 320 ng Dienogest/L (nominal), respective 31.7, 105 and 335 ng Dienogest/L (mean measured); (based on histological data provided in C.1.14)

	Nominal concentration Dienogest [ng/L]											
	32.0				100				320			
	Mean measured concentration Dienogest [ng/L]											
	31.7				105				335			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Maturation stage females**											
1	-	3	3	2		3	3	2	3	3	3	3
2	3	3	3	-		3	3	3	3	3	2	-
3	-	3	3	3		2	3	3	3	3	-	3
4	3	-	-	3		3	-	-	-	2	3	-
5	-	-	3	-		-	-	2	3	2	-	3
6	3	3	2	-		-	-	-	-	3	-	2
7	-	-	-	2		-	-	3	-	-	-	3
8	-	-	-	-		3	-	2	-	-	-	-
9	3	-	-	3		3	-	3	-	-	-	-
10	-	-	2	3		2	-	2	-	-	-	-
11	2	3	3	3		3	-	-	-	-	-	-
12	-	-	3	3		3	-	-	-	-	-	3
13	-	-	3	3		-	-	3	-	-	4	2
14	-	-	-	3		-	-	3	-	-	3	-
15	3	-	3	3		-	-	3	-	-	3	-
16	3	-	3	3		3	-	3	-	-	2	-
17	-	-	3	-		3	-	-	-	-	-	-
18	2	-	-	-		-	-	-	-	-	3	-
19	-	-	-	-		-	-	-	-	-	-	-
20	-	-	-	-		-	-	-	-	-	2	-
Median	**3**	**3**	**3**	**3**	**-**	**3**	**3**	**3**	**3**	**3**	**3**	**3**

Table 101: F_0 generation, maturation stages, summary

		Nominal concentration Dienogest [ng/L]					
		control	3.20	10.0	32.0	100	320
		Mean measured concentration Dienogest [ng/L]					
	Replicate	Control	3.51	10.3	31.7	105	335
Maturation stage males, median value	A	2	2	2	2	-	2
	B	2	2	2	2	2	2
	C	2	2	2	2	3	2
	D	2	2	2	2	2	2
Maturation stage females, median value	A	2	3	3	3	-	3
	B	3	3	3	3	3	3
	C	3	3	3	3	3	3
	D	3	3	3	3	3	3

Table 102: F_1 generation, testis-ova, males, control, 3.20 and 10.0 ng Dienogest/L (nominal), respective 3.51 and 10.3 ng Dienogest/L (mean measured); (based on histological data provided in C.1.14)

	Nominal concentration Dienogest [ng/L]											
	control				**3.20**				**10.0**			
	Mean measured concentration Dienogest [ng/L]											
	Control				**3.51**				**10.3**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Testis-ova, males**											
1	0	-	-	-	0	0	-	1	-	-	-	-
2	0	-	-	0	0	-	1	0	-	0	0	-
3	0	-	-	-	-	-	0	0	-	0	-	-
4	1	-	0	-	-	-	0	0	-	-	0	-
5	-	-	0	1	0	0	0	0	-	0	1	-
6	0	0	0	0	-	-	0	-	0	0	0	-
7	0	0	0	2	-	-	0	-	0	-	0	-
8	-	0	0	1	0	-	-	0	-	-	0	0
9	-	1	0	-	-	-	-	-	-	-	-	0
10	0	0	0	-	0	-	-	-	-	-	-	0
11	1	-	0	-	-	-	0	-	-	0	-	0
12	0	0	-	0	0	-	0	-	0	0	-	-
13	-	-	-	0	0	0	0	0	-	-	1	0
14	0	-	0	-	-	0	-	-	-	-	1	0
15	-	-	-	0	0	0	-	-	0	0	-	-
16	-	-	0	0	0	0	0	0	0	-	0	0
17	-	0	0	0	0	1	0	-	-	0	0	-
18	0	0	0	0	-	0	0	0	-	0	-	-
19	0	-	0	0	-	0	0	0	-	-	0	1
20	0	0	-	0	0	-	0	0	0	-	0	-
Median	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table 103: F_1 generation, testis-ova, males, 32.0, 100 and 320 ng Dienogest/L (nominal), respective 31.7, 105 and 335 ng Dienogest/L (mean measured); (based on histological data provided in C.1.14)

	Nominal concentration Dienogest [ng/L]											
	32.0				100				320			
	Mean measured concentration Dienogest [ng/L]											
	31.7				105				335			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Testis-ova, males**											
1	0	-	-	-		-	-	-	-	-	-	-
2	-	-	-	0		-	-	-	-	-	-	0
3	0	-	-	-		-	-	-	-	-	0	-
4	-	0	0	-		-	2	0	0	-	-	0
5	0	0	-	0		0	0	-	-	-	0	-
6	-	-	-	0		0	0	1	0	-	0	-
7	0	0	0	-		0	0	-	0	0	0	-
8	0	0	0	0		-	-	-	0	0	0	0
9	-	0	0	-		-	-	-	0	0	0	0
10	0	0	-	-		-	-	-	0	-	0	0
11	-	-	-	-		-	-	0	0	-	0	0
12	0	0	-	-		-	-	0	-	-	0	-
13	0	0	-	-		0	-	-	-	-	-	-
14	0	0	0	-		0	-	-	-	-	-	0
15	-	0	-	-		0	-	-	-	-	-	0
16	-	0	-	-		-	-	-	-	-	-	0
17	0	-	-	0		-	-	0	-	-	4	0
18	-	-	0	0		0	-	-	-	-	-	0
19	0	-	0	0		0	-	-	-	-	0	-
20	0	-	-	1		-	-	-	-	-	-	-
Median	**0**	**0**	**0**	**0**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table 104: F_1 generation, increased oocyte atresia, females, control, 3.20 and 10.0 ng Dienogest/L (nominal), respective 3.51 and 10.3 ng Dienogest/L (mean measured); (based on histological data provided in C.1.14)

	Nominal concentration Dienogest [ng/L]											
	control				3.20				10.0			
	Mean measured concentration Dienogest [ng/L]											
	Control				3.51				10.3			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Increased oocyte atresia, females											
1	-	0	0	0	-	-	0	-	0	0	0	0
2	-	0	0	-	-	0	-	-	0	-	-	0
3	-	1	0	0	0	0	-	-	0	-	0	0
4	-	0	-	0	0	0	-	-	0	0	-	0
5	0	0	-	-	-	-	-	-	0	-	-	0
6	-	-	-	-	0	0	-	0	-	-	-	0
7	-	-	-	-	0	0	-	0	-	0	-	0
8	0	-	-	-	-	0	0	-	0	0	-	-
9	0	-	-	0	0	0	0	0	0	0	0	-
10	-	-	-	0	-	0	0	0	0	0	0	-
11	-	0	-	0	0	0	-	0	0	-	0	-
12	-	-	0	-	-	0	-	0	-	-	0	0
13	0	0	0	-	-	-	-	-	0	0	-	-
14	-	0	-	0	0	-	0	0	0	0	-	-
15	0	0	0	-	-	-	0	0	-	-	0	0
16	0	0	-	-	-	-	-	-	-	0	-	-
17	0	-	-	-	-	-	-	0	0	-	-	0
18	-	-	-	-	0	-	-	-	0	-	0	0
19	-	0	-	-	0	-	-	-	0	0	-	-
20	-	-	-	-	-	-	-	-	-	0	-	0
Median	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table 105: F_1 generation, increased oocyte atresia, females, 32.0, 100 and 320 ng Dienogest/L (nominal), respective 31.7, 105 and 335 ng Dienogest/L (mean measured); (based on histological data provided in C.1.14)

	Nominal concentration Dienogest [ng/L]											
	32.0				100				320			
	Mean measured concentration Dienogest [ng/L]											
	31.7				105				335			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Increased oocyte atresia, females**											
1	-	0	0	0		0	0	0	0	0	0	0
2	0	0	0	-		0	0	0	0	0	0	-
3	-	0	0	0		0	0	0	0	1	-	0
4	0	-	-	0		0	-	-	-	0	0	-
5	-	-	0	-		-	-	0	0	0	-	0
6	0	0	0	-		-	-	-	-	0	-	0
7	-	-	-	0		-	-	0	-	-	-	0
8	-	-	-	-		0	-	0	-	-	-	-
9	0	-	-	0		0	-	0	-	-	-	-
10	-	-	0	0		0	-	0	-	-	-	-
11	0	0	0	0		0	-	-	-	-	-	-
12	-	-	0	0		0	-	-	-	-	-	0
13	-	-	0	0		-	-	0	-	-	1	0
14	-	-	-	0		-	-	0	-	-	0	-
15	0	-	0	0		-	-	0	-	-	1	-
16	0	-	0	0		0	-	0	-	-	0	-
17	-	-	0	-		0	-	-	-	-	-	-
18	0	-	-	-		-	-	-	-	-	0	-
19	-	-	-	-		-	-	-	-	-	-	-
20	-	-	-	-		-	-	-	-	-	0	-
Median	**0**	**0**	**0**	**0**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table 106: **F_1 generation, egg debris, females, control, 3.20 and 10.0 ng Dienogest/L (nominal), respective 3.51 and 10.3 ng Dienogest/L (mean measured); (based on histological data provided in C.1.14)**

	Nominal concentration Dienogest [ng/L]											
	Control				3.20				10.0			
	Mean measured concentration Dienogest [ng/L]											
	Control				3.51				10.3			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Egg debris, females											
1	-	0	1	2	-	-	0	-	0	0	0	0
2	-	0	0	-	-	0	-	-	0	-	-	0
3	-	0	1	0	0	0	-	-	1	-	0	0
4	-	0	-	0	0	0	-	-	0	0	-	0
5	0	0	-	-	-	-	-	-	0	-	-	0
6	-	-	-	-	1	0	-	2	-	-	-	0
7	-	-	-	-	0	0	-	0	-	0	-	0
8	0	-	-	-	-	0	0	-	0	0	-	-
9	0	-	-	1	0	0	0	1	0	0	0	-
10	-	-	-	0	-	0	0	0	0	0	0	-
11	-	0	-	0	0	0	-	0	0	-	0	-
12	-	-	0	-	-	0	-	0	-	-	0	0
13	0	1	0	-	-	-	-	-	0	0	-	-
14	-	0	-	0	0	-	0	0	0	0	-	-
15	0	0	0	-	-	-	0	1	-	-	0	0
16	0	0	-	-	-	-	-	-	-	0	-	-
17	0	-	-	-	-	-	-	0	0	-	-	0
18	-	-	-	-	0	-	-	-	0	-	1	0
19	-	0	-	-	2	-	-	-	0	0	-	-
20	-	-	-	-		-	-	-	-	0	-	0
Median	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table 107: F_1 generation, egg debris, females, 32.0, 100 and 320 ng Dienogest/L (nominal), respective 31.7, 105 and 335 ng Dienogest/L (mean measured); (based on histological data provided in C.1.14)

	Nominal concentration Dienogest [ng/L]											
	32.0				100				320			
	Mean measured concentration Dienogest [ng/L]											
	31.7				105				335			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Egg debris, females											
1	-	0	0	0		0	0	0	0	0	0	0
2	0	0	0	-		0	0	0	0	2	0	-
3	-	1	0	0		0	2	0	0	0	-	0
4	0	-	-	0		2	-	-	-	1	0	-
5	-	-	1	-		-	-	0	0	0	-	1
6	0	0	0	-		-	-	-	-	0	-	1
7	-	-	-	0		-	-	0	-	-	-	0
8	-	-	-	-		2	-	0	-	-	-	-
9	0	-	-	0		0	-	0	-	-	-	-
10	-	-	0	0		0	-	0	-	-	-	-
11	0	0	1	0		0	-	-	-	-	-	-
12	-	-	0	0		0	-	-	-	-	-	0
13	-	-	0	0		-	-	0	-	-	4	1
14	-	-	-	0		-	-	0	-	-	0	-
15	0	-	0	0		-	-	0	-	-	2	-
16	0	-	0	0		0	-	0	-	-	0	-
17	-	-	0	-		0	-	-	-	-	-	-
18	0	-	-	-		-	-	-	-	-	0	-
19	-	-	-	-		-	-	-	-	-	-	-
20	-	-	-	-		-	-	-	-	-	0	-
Median	**0**	**0**	**0**	**0**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table 108: F_1 generation, granulomatous inflammation, females, control, 3.20 and 10.0 ng Dienogest/L (nominal), respective 3.51 and 10.3 ng Dienogest/L (mean measured); (based on histological data provided in C.2.14)

	Nominal concentration Dienogest [ng/L]											
	Control				**3.20**				**10.0**			
	Mean measured concentration Dienogest [ng/L]											
	Control				**3.51**				**10.3**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Granulomatous inflammation, females**											
1	-	0	1	0	-	-	0	-	1	0	0	0
2	-	0	0	-	-	0	-	-	1	-	-	0
3	-	0	0	0	0	0	-	-	0	-	0	0
4	-	0	-	0	0	0	-	-	0	0	-	0
5	0	0	-	-	-	-	-	-	0	-	-	0
6	-	-	-	-	0	0	-	1	-	-	-	0
7	-	-	-	-	0	0	-	0	-	0	-	0
8	0	-	-	-	-	0	0	-	0	0	-	-
9	0	-	-	2	0	0	0	0	0	1	0	-
10	-	-	-	0	-	0	0	0	0	0	0	-
11	-	0	-	0	0	0	-	0	0	-	0	-
12	-	-	0	-	-	0	-	0	-	-	0	0
13	0	0	1	-	-	-	-	-	0	0	-	-
14	-	0	-	0	0	-	0	0	0	0	-	-
15	0	0	0	-	-	-	0	0	-	-	0	0
16	0	0	-	-	-	-	-	-	-	0	-	-
17	0	-	-	-	-	-	-	0	0	-	-	0
18	-	-	-	-	1	-	-	-	0	-	0	0
19	-	0	-	-	1	-	-	-	0	0	-	-
20	-	-	-	-	-	-	-	-	-	0	-	0
Median	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table 109: F_1 generation, granulomatous inflammation, females, 32.0, 100 and 320 ng Dienogest/L (nominal), respective 31.7, 105 and 335 ng Dienogest/L (mean measured); (based on histological data provided in C.1.14)

	Nominal concentration Dienogest [ng/L]											
	32.0				100				320			
	Mean measured concentration Dienogest [ng/L]											
	31.7				105				335			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Granulomatous inflammation, females**											
1	-	0	0	0	-	0	0	0	0	0	0	0
2	0	0	0	-		0	0	0	0	1	0	-
3	-	0	0	0		0	1	0	0	0	-	0
4	0	-	-	0		0	-	-	-	0	0	-
5	-	-	0	-		-	-	0	0	0	-	0
6	0	0	0	-		-	-	-	-	0	-	0
7	-	-	-	0		-	-	0	-	-	-	0
8	-	-	-	-		0	-	0	-	-	-	-
9	0	-	-	0		0	-	0	-	-	-	-
10	-	-	1	0		0	-	0	-	-	-	-
11	0	0	0	0		0	-	-	-	-	-	-
12	-	-	0	1		0	-	-	-	-	-	0
13	-	-	0	0		-	-	0	-	-	3	0
14	-	-	-	0		-	-	0	-	-	0	-
15	0	-	0	1		-	-	0	-	-	2	-
16	0	-	0	0		0	-	0	-	-	0	-
17	-	-	0	-		1	-	-	-	-	-	-
18	0	-	-	-		-	-	-	-	-	0	-
19	-	-	-	-		-	-	-	-	-	-	-
20	-	-	-	-		-	-	-	-	-	0	-
Median	**0**	**0**	**0**	**0**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table 110: F_1 generation, increased post-ovulatory follicles, females, control, 3.20 and 10.0 ng Dienogest/L (nominal), respective 3.51 and 10.3 ng Dienogest/L (mean measured); (based on histological data provided in C.2.14)

	Nominal concentration Dienogest [ng/L]											
	Control				3.20				10.0			
	Mean measured concentration Dienogest [ng/L]											
	Control				3.51				10.3			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Increasded post-ovulatory follicles, females**											
1	-	0	1	0	-	-	0	-	0	1	0	0
2	-	0	0	-	-	0	-	-	0	-	-	0
3	-	0	0	0	0	0	-	-	0	-	0	0
4	-	0	-	0	0	0	-	-	0	2	-	0
5	1	0	-	-	-	-	-	-	0	-	-	0
6	-	-	-	-	0	1	-	1	-	-	-	0
7	-	-	-	-	0	0	-	0	-	0	-	0
8	0	-	-	-	-	0	0	-	0	0	-	-
9	0	-	-	0	0	0	0	0	0	0	0	-
10	-	-	-	0	-	0	0	0	0	0	2	-
11	-	0	-	0	0	0	-	0	0	-	0	-
12	-	-	0	-	-	0	-	0	-	-	0	0
13	0	0	0	-	-	-	-	-	0	2	-	-
14	-	1	-	0	0	-	1	0	0	0	-	-
15	0	0	0	-	-	-	0	0	-	-	0	0
16	0	0	-	-	-	-	-	-	-	0	-	-
17	0	-	-	-	-	-	-	1	0	-	-	0
18	-	-	-	-	0	-	-	-	0	-	0	0
19	-	0	-	-	0	-	-	-	0	0	-	-
20	-	-	-	-	-	-	-	-	-	0	-	0
Median	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table 111: F_1 generation, increased post-ovulatory follicles, females, 32.0, 100 and 320 ng Dienogest/L (nominal), respective 31.7, 105 and 335 ng Dienogest/L (mean measured); (based on histological data provided in C.2.14)

	Nominal concentration Dienogest [ng/L]											
	32.0				100				320			
	Mean measured concentration Dienogest [ng/L]											
	31.7				105				335			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Increased post-ovulatory follicles, females											
1	-	1	0	2		0	0	1	0	0	0	0
2	0	0	0	-		0	1	0	0	1	1	-
3	-	0	0	0		1	0	1	0	0	-	0
4	0	-	-	2		0	-	-	-	1	0	-
5	-	-	0	-		-	-	1	0	0	-	2
6	0	0	0	-		-	-	-	-	0	-	2
7	-	-	-	0		-	-	0	-	-	-	0
8	-	-	-	-		0	-	0	-	-	-	-
9	0	-	-	0		0	-	0	-	-	-	-
10	-	-	0	0		1	-	2	-	-	-	-
11	0	0	0	0		0	-	-	-	-	-	-
12	-	-	0	0		0	-	-	-	-	-	1
13	-	-	0	0		-	-	0	-	-	0	0
14	-	-	-	0		-	-	0	-	-	0	-
15	0	-	0	0		-	-	0	-	-	2	-
16	0	-	0	0		0	-	0	-	-	0	-
17	-	-	0	-		0	-	-	-	-	-	-
18	1	-	-	-		-	-	-	-	-	0	-
19	-	-	-	-		-	-	-	-	-	-	-
20	-	-	-	-		-	-	-	-	-	0	-
Median	**0**	**0**	**0**	**0**	**-**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Table 112: F_1 generation, grading of lesions severity, summary

		Nominal concentration Dienogest [ng/L]					
		Control	3.20	10.0	32.0	100	320
		Mean measured concentration Dienogest [ng/L]					
	Replicate	Control	3.51	10.3	31.7	105	335
Testis-ova, males, median value	A	0	0	0	0	-	0
	B	0	0	0	0	0	0
	C	0	0	0	0	0	0
	D	0	0	0	0	0	0
Increased oocyte atresia, females, median value	A	0	0	0	0	-	0
	B	0	0	0	0	0	0
	C	0	0	0	0	0	0
	D	0	0	0	0	0	0
Egg debris, females, median value	A	0	0	0	0	-	0
	B	0	0	0	0	0	0
	C	0	0	0	0	0	0
	D	0	0	0	0	0	0
Granulomatous inflammation, females, median value	A	0	0	0	0	-	0
	B	0	0	0	0	0	0
	C	0	0	0	0	0	0
	D	0	0	0	0	0	0
Increased post-ovulatory follicles, females, median value	A	0	0	0	0	-	0
	B	0	0	0	0	0	0
	C	0	0	0	0	0	0
	D	0	0	0	0	0	0

C.1.15 Statistical evaluation, treatment with Dienogest, F_0 generation

All endpoints were statistically evaluated with ToxRat (ToxRat® Professional 3.3.0; ToxRat® Solutions GmbH, Alsdorf, Germany). For the biological parameters recorded for F_0 generation, no significant differences between control and treatment with Dienogest could be determined.

C.1.16 Statistical evaluation, treatment with Dienogest, F_1 generation

All biological endpoints were statistically evaluated with ToxRat (ToxRat® Professional 3.3.0; ToxRat® Solutions GmbH, Alsdorf, Germany). Only statistically significant results are presented in the following. Please note, that the nominal concentrations are given.

► **Post hatch survival [%], day 21 pf, first filial generation (F_1)**

Data were arcsine transformed prior to evaluation.

Table 113: F_1 post hatch survival of *Danio rerio* as dependent on concentration of the test item and time (day 21 pf)

Treatm. [ng/L]	Control	3.2	10.0	32.0	100.0	320.0
	1.278	1.080	1.231	0.985	0.000	0.615
	1.231	1.150	0.955	1.189	1.189	0.524
	1.333	1.114	0.869	0.841	0.457	1.150
	1.403	1.114	0.985	1.114	0.758	0.785
Mean	**1.311**	**1.115**	**1.010**	**1.032**	**0.601**	**0.769**
Std.Dev.	0.0742	0.0287	0.1552	0.1528	0.5007	0.2765
n	4	4	4	4	4	4
CV	5.7	2.6	15.4	14.8	83.3	36.0

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from Tabs F1 Post hatch survival)

The normality check was passed ($p > 0.01$). The Levene test indicated variance homogeneity ($p > 0.010$). Thus, the variance homogeneity check was passed. As normal-distribution and variance-homogeneity requirements were fulfilled, a parametric multiple test was advisable.

The analysis of contrasts revealed a linear trend; thus, the Williams test was performed.

Table 114: Williams Multiple Sequential t-test Procedure (post hatch survival at day 21 pf, F_1)

Treatm. [ng/L]	Mean	s	df	LhM	%MDD	t	t*	Sign
Control	1.311	0.25198						
3.2	1.115	0.25198	18	1.115	-23.6	-1.10	-1.73	-
10.0	1.010	0.25198	18	1.021	-24.7	-1.63	-1.82	-
32.0	1.032	0.25198	18	1.021	-25.1	-1.63	-1.85	-
100.0	**0.601**	**0.25198**	**18**	**0.685**	**-25.3**	**-3.52**	**-1.86**	**+**
320.0	**0.769**	**0.25198**	**18**	**0.685**	**-25.4**	**-3.52**	**-1.87**	**+**

+: significant; -: non-significant

Comparison of treatments with "Control" by the t test procedure after Williams with F1 post hatch survival at day 21 pf: Significance was Alpha = 0.050. one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: μ1 = μ2 = ... = μk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered.

Based on this, a NOEC of 32.0 ng Dienogest/L was determined. This is corresponding to the mean measured concentration of 31.7 ng Dienogest/L.

► Post-hatch survival [%], day 35 pf, first filial generation (F_1)

Data were arcsine transformed prior to evaluation.

Table 115: F_1 post hatch survival of *Danio rerio* as dependent on concentration of the test item and time (day 35 pf)

Treatm. [ng/L]	Control	3.2	10.0	32.0	100.0	320.0
	1.278	1.080	1.231	0.955	0.000	0.615
	1.189	1.150	0.955	1.189	1.150	0.524
	1.333	1.080	0.869	0.813	0.457	1.114
	1.403	1.080	0.985	1.114	0.758	0.785
Mean	**1.301**	**1.098**	**1.010**	**1.018**	**0.591**	**0.760**
Std.Dev.	0.0905	0.0352	0.1552	0.1677	0.4857	0.2600
n	4	4	4	4	4	4
CV	7.0	3.2	15.4	16.5	82.2	34.2

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from Tabs F1 Post hatch survival, day 35 pf)

The normality check was passed (p > 0.01). The Levene test indicated variance homogeneity (p > 0.010). Thus, the variance homogeneity check was passed. As normal-distribution and variance-homogeneity requirements were fulfilled, a parametric multiple test was advisable.

The analysis of contrasts revealed a linear trend; thus, the Williams test was performed.

Table 116: Williams Multiple Sequential t-test Procedure (post hatch survival at day 35 pf, F_1)

Treatm. [ng/L]	Mean	s	df	LhM	%MDD	t	t*	Sign
Control	1.301	0.24671						
3.2	1.098	0.24671	18	1.098	-23.3	-1.17	-1.73	-
10.0	1.010	0.24671	18	1.014	-24.4	-1.64	-1.82	-
32.0	1.018	0.24671	18	1.014	-24.7	-1.64	-1.85	-
100.0	**0.591**	**0.24671**	**18**	**0.675**	**-24.9**	**-3.58**	**-1.86**	**+**
320.0	**0.760**	**0.24671**	**18**	**0.675**	**-25.0**	**-3.58**	**-1.87**	**+**

+: significant; -: non-significant

Comparison of treatments with "Control" by the t test procedure after Williams with F1 post hatch survival, day 35 pf: Significance was Alpha = 0.050. one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: μ1 = μ2 = ... = μk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered.

Based on this, a NOEC of 32.0 ng Dienogest/L was determined. This is corresponding to the mean measured concentration of 31.7 ng Dienogest/L.

► Total length [cm], day 35 pf, first filial generation (F1)

Table 117: Total length [cm] of *Danio rerio* as dependent on concentration of the test item and time (day 35 pf)

Treatm. [ng/L]	Control	3.2	10.0	32.0	100.0	320.0
	1.8	1.8	1.7	1.8	-	2.1
	1.9	1.8	1.7	1.8	1.7	2.1
	1.9	1.8	1.8	1.8	2.1	1.8
	1.8	1.8	1.8	1.8	1.8	1.8
Mean	**1.9**	**1.8**	**1.7**	**1.8**	**1.9**	**1.9**
Std.Dev.	0.05	0.02	0.05	0.01	0.20	0.17
n	4	4	4	4	3	4
CV	2.6	1.0	2.8	0.5	10.8	8.7

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from Tabs Total lengtht day 35 pf)

The normality check was passed ($p > 0.01$). The Levene test indicated variance heterogeneity ($p \leq 0.010$). However, normal distribution requirements were fulfilled.

The Welch-t-test for non-homogeneous variances with Bonferroni-Holm-adjustment was advisable.

Table 118: Multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm (total length, day 35 pf, F_1)

Treatm. [ng/L]	Mean	s	df	%MDD	t	P(t)	Alpha(i)	Sign
Control	1.9	0.05						
3.2	1.8	0.02	3	-5.8	-2.96	0.030	0.013	-
10.0	**1.7**	**0.05**	**5**	**-6.1**	**-3.67**	**0.007**	**0.010**	**+**
32.0	1.8	0.01	3	-4.9	-2.80	0.034	0.017	-
100.0	1.9	0.20	2	-27.8	0.26	0.589	0.025	-
320.0	1.9	0.17	3	-11.2	1.08	0.820	0.050	-

Multiple sequentially rejective comparisons of treatments with "Control". Significance was Alpha = 0.050, one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; p(t): probability of sample t for Ho: $\mu 1 = \mu 2$; Alpha(i): adjusted significance levels; the differences are significant in case p(t) <= Alpha(i); dfm: modified degrees of freedom due to heteroscedasticity.(Control(c) and treatment(t) variance was applied: $s^2(c)/nc + s^2(t)/nt$, each). Note that the step-down test terminates after the first non-significant treatment is encountered.

Fertility, first filial generation (F_1)

Data were arcsine transformed prior evaluation.

Table 119: Fertilisation rate of *Danio rerio* as dependent on concentration of the test item and time (F_1)

Treatm. [ng/L]	Control	3.2	10.0	32.0	100.0	320.0
	1.246	1.260	1.227	1.204	-	0.847
	1.124	1.295	1.249	1.147	1.294	0.994
	1.198	1.190	1.257	1.133	0.951	1.098
	1.214	1.185	1.173	1.161	1.143	1.141
Mean	**1.195**	**1.233**	**1.227**	**1.161**	**1.129**	**1.020**
Std.Dev.	0.0515	0.0538	0.0382	0.0308	0.1717	0.1309
n	4	4	4	4	3	4
CV	4.3	4.4	3.1	2.7	15.2	12.8

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from Tabs F1 Fertilisation rate).

The normality check was passed ($p > 0.01$). The Levene test indicated variance homogeneity ($p > 0.010$). Thus, the variance homogeneity check was passed. As normal-distribution and variance-homogeneity requirements were fulfilled, a parametric multiple test was advisable.

The analysis of contrasts revealed a linear trend; thus, the Williams test was performed. Trend analysis by Contrasts (Monotonicity of Concentration/Response)

Table 120: Williams Multiple Sequential t-test Procedure (fertilisation rate, F_1)

Treatm. [ng/L]	Mean	s	df	LhM	%MDD	t	t*	Sign
Control	1.195	0.08884						
3.2	1.233	0.08884	17	1.233	-9.1	0.59	-1.74	-
10.0	1.227	0.08884	17	1.227	-9.6	0.50	-1.82	-
32.0	1.161	0.08884	17	1.161	-9.7	-0.54	-1.85	-
100.0	1.129	0.08884	17	1.129	-10.5	-0.97	-1.85	-
320.0	**1.020**	**0.08884**	**17**	**1.020**	**-9.8**	**-2.79**	**-1.87**	**+**

+: significant; -: non-significant; Comparison of treatments with "Control" by the t test procedure after Williams with f1 fertilisation rate at Test end: Significance was Alpha = 0.050. one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: $\mu1 = \mu2 = ... = \mu k$; the differences are significant in case $|t| > |t^*|$ (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered.

Based on this, a NOEC of 100 ng Dienogest/L was determined. This is corresponding to the mean measured concentration of 105 ng Dienogest/L.

C.1.17 Statistics, treatment with Dienogest, F_2 generation

Please note, that the nominal concentrations are given.

► **Hatching rate [%], data arcsine transformed prior to evaluation**

Table 121: F_2 hatching rate [%] of *Danio rerio* as dependent on concentration of the test item and time (data arcsine transformed prior to evaluation)

Treatm. [ng/L]	Control	3.2	10.0	32.0	100.0	320.0
	1.047	1.249	1.173	0.785	-	0.226
	1.107	1.107	0.464	0.633	0.735	0.580
	1.571	0.938	0.785	0.633	0.938	0.785
	1.107	0.938	0.886	0.938	0.886	1.047
Mean	**1.208**	**1.058**	**0.827**	**0.747**	**0.853**	**0.659**
Std.Dev.	0.2435	0.1504	0.2927	0.1459	0.1052	0.3468
n	4	4	4	4	3	4
CV	20.2	14.2	35.4	19.5	12.3	52.6

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from Tabs F2 Hatching)

The normality check was passed ($p > 0.01$). The Levene test indicated variance homogeneity ($p > 0.010$). Thus, the variance homogeneity check was passed. As normal-distribution and variance-homogeneity requirements were fulfilled, a parametric multiple test was advisable.

The analysis of contrasts revealed a linear trend; thus, the Williams test was performed.

Table 122: Williams Multiple Sequential t-test Procedure (hatching rate, F_2)

Treatm. [ng/L]	Mean	s	df	LhM	%MDD	t	t*	Sign
Control	Control	1.208	0.23633					
3.2	1.058	0.23633	17	1.058	-24.1	-0.90	-1.74	-
10.0	**0.827**	**0.23633**	**17**	**0.827**	**-25.2**	**-2.28**	**-1.82**	**+**
32.0	**0.747**	**0.23633**	**17**	**0.793**	**-25.6**	**-2.49**	**-1.85**	**+**
100.0	**0.853**	**0.23633**	**17**	**0.793**	**-27.7**	**-2.30**	**-1.85**	**+**
320.0	**0.659**	**0.23633**	**17**	**0.659**	**-25.9**	**-3.28**	**-1.87**	**+**

+: significant; -: non-significant

Comparison of treatments with "Control" by the t test procedure after Williams with f2 hatching/survival at Test end: Significance was Alpha = 0.050. one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: μ1 = μ2 = ... = μk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered

Based on this, a NOEC of 3.20 ng Dienogest/L was determined. This is corresponding to the mean measured concentration of 3.51 ng Dienogest/L.

C.2 Ecotoxicological Studies with Dexamethasone: Zebrafish extended one generation reproduction test (ZEOGRT)

C.2.1 Test conditions: water temperature

Table 123: Water temperature [°C]; control and 0.32 µg Dexamethasone/L

	Nominal concentration Dexamethasone [µg/L]							
	Control				0.32			
Replicate	A	B	C	D	A	B	C	D
Time of exposure [day]	Water temperature [°C]							
0	25.9	25.8	26.3	26.3	25.6	25.7	26.1	26.1
1	25.9	25.8	26.4	26.3	25.7	25.7	26.1	26.1
4	26.8	26.8	27.1	27.1	26.7	26.7	27.1	27.1
5	26.8	26.8	27.1	27.1	26.7	26.7	27.1	27.1
6	26.6	26.6	26.9	26.9	26.4	26.5	26.9	26.9
7	26.6	26.5	26.8	26.9	26.4	26.5	26.8	26.9
8	26.6	26.5	26.8	26.9	26.4	26.5	26.8	26.9
11	26.6	26.6	26.7	26.9	26.5	26.5	26.8	26.8
12	26.7	26.7	26.8	26.9	26.6	26.6	26.8	26.9
13	26.6	26.6	26.8	26.8	26.5	26.5	26.7	26.8
14	26.4	26.5	26.7	26.8	26.5	26.5	26.8	26.7
15	26.4	26.4	26.7	26.7	26.5	26.5	26.7	26.6
18	26.5	26.5	26.8	26.8	26.4	26.4	26.7	26.7
19	26.5	26.5	26.8	26.8	26.4	26.4	26.7	26.7
20	26.4	26.4	26.7	26.7	26.3	26.3	26.7	26.7
21	26.4	26.4	26.7	26.7	26.3	26.3	26.7	26.7
22	26.5	26.5	26.7	26.8	26.4	26.4	26.8	26.8
25	26.5	26.5	26.8	26.8	26.4	26.4	26.8	26.8
26	26.4	26.4	26.6	26.6	26.3	26.3	26.6	26.6
27	26.4	26.4	26.6	26.6	26.3	26.3	26.5	26.5
28	26.6	26.6	26.8	26.8	26.4	26.4	26.7	26.7
29	26.6	26.6	26.8	26.7	26.4	26.3	26.7	26.7
32	26.3	26.3	26.5	26.6	26.2	26.2	26.5	26.5
33	26.4	26.4	26.5	26.6	26.3	26.3	26.6	26.6

	Nominal concentration Dexamethasone [µg/L]							
34	26.4	26.4	26.5	26.6	26.3	26.3	26.6	26.6
35	26.3	26.3	26.5	26.5	26.2	26.2	26.5	26.5
36	26.2	26.2	26.4	26.5	26.1	26.0	26.5	26.5
39	26.4	26.4	26.6	26.6	26.3	26.3	26.5	26.5
40	26.4	26.4	26.6	26.7	26.3	26.3	26.5	26.5
41	26.4	26.4	26.6	26.7	26.3	26.2	26.5	26.5
42	26.4	26.4	26.6	26.6	26.3	26.3	26.5	26.5
43	26.3	26.3	26.5	26.5	26.1	26.1	26.3	26.4
46	26.4	26.4	26.5	26.6	26.2	26.2	26.3	26.5
47	26.4	26.4	26.5	26.6	26.2	26.2	26.3	26.4
48	26.1	26.1	26.3	26.4	26.0	26.0	26.3	26.3
49	26.2	26.2	26.3	26.5	26.1	26.1	26.3	26.3
50	26.3	26.4	26.4	26.5	26.2	26.2	26.3	26.3
53	26.3	26.3	26.4	26.5	26.1	26.1	26.3	26.3
54	26.2	26.2	26.3	26.4	26.0	26.0	26.2	26.2
55	26.2	26.1	26.4	26.4	26.0	26.0	26.4	26.3
56	26.3	26.2	26.5	26.5	26.0	26.0	26.4	26.4
57	26.3	26.2	26.4	26.6	26.0	26.0	26.3	26.3
60	26.2	26.1	26.4	26.4	26.0	26.0	26.2	26.2
61	26.0	26.1	26.1	26.4	25.9	26.0	26.2	26.2
62	26.2	26.0	26.2	26.3	26.0	26.0	26.2	26.2
63	26.2	26.2	26.2	26.4	26.1	26.2	26.2	26.2
64	26.2	26.2	26.2	26.3	26.0	26.0	26.1	26.2
67	26.1	26.1	26.0	26.2	26.0	25.9	26.0	26.0
68	26.2	26.3	26.1	26.3	26.1	25.9	26.1	26.1
69	26.3	26.3	26.2	26.4	26.0	25.9	26.2	26.3
70	26.3	26.3	26.2	26.3	26.1	26.0	26.2	26.3
71	26.2	26.2	26.1	26.2	26.0	25.9	26.1	26.2
74	26.1	26.1	25.9	26.0	25.9	25.8	25.9	26.0
75	26.1	26.1	26.0	26.0	25.9	25.8	25.9	25.9
76	26.1	26.2	26.1	26.2	25.9	25.8	26.1	26.2
77	26.0	26.1	26.0	26.2	26.0	25.9	26.1	26.2

	Nominal concentration Dexamethasone [µg/L]							
78	26.2	26.2	26.0	26.2	25.9	25.8	26.0	26.1
81	26.3	26.4	26.1	26.2	26.0	25.9	26.1	26.3
82	26.4	26.5	26.1	26.2	26.1	26.0	26.1	26.3
83	26.4	26.5	26.2	26.2	26.1	26.0	26.1	26.3
84	26.5	26.5	26.3	26.4	26.2	26.2	26.3	26.3
85	26.5	26.5	26.4	26.5	26.3	26.2	26.3	26.4
88	26.5	26.5	26.3	26.4	26.3	26.3	26.2	26.3
89	26.5	26.4	26.2	26.3	26.2	26.2	26.3	26.3
90	26.5	26.4	26.2	26.3	26.2	26.2	26.2	26.3
91	26.4	26.3	26.2	26.2	26.1	26.1	26.1	26.2
92	26.5	26.4	26.2	26.3	26.2	26.2	26.2	26.2
95	26.4	26.5	26.2	26.3	26.3	26.4	26.1	26.3
96	26.3	26.5	26.4	26.3	26.2	26.2	26.2	26.3
97	26.3	26.4	26.4	26.3	26.2	26.2	26.2	26.3
98	26.6	26.6	26.5	26.6	26.4	26.3	26.3	26.5
99	26.5	26.6	26.5	26.4	26.3	26.2	26.2	26.3
102	26.7	26.6	26.5	26.4	26.3	26.2	26.3	26.4
103	26.7	26.6	26.5	26.5	26.3	26.3	26.3	26.4
104	26.5	26.3	26.3	26.3	26.3	26.2	26.3	26.4
105	26.4	26.3	26.3	26.3	26.3	26.2	26.2	26.3
106	26.4	26.3	26.3	26.3	26.2	26.2	26.2	26.3
109	26.4	26.3	26.3	26.3	26.2	26.1	26.1	26.2
110	26.5	26.3	26.2	26.2	26.2	26.1	26.1	26.2
111	26.5	26.3	26.3	26.3	26.2	26.2	26.1	26.2
112	26.4	26.3	26.4	26.4	26.2	26.1	26.1	26.2
113	26.5	26.4	26.5	26.6	26.2	26.2	26.2	26.3
116	26.5	26.4	26.5	26.5	26.2	26.2	26.2	26.2
117	26.5	26.3	26.5	26.4	26.1	26.1	26.1	26.1
118	26.5	26.3	26.5	26.5	26.2	26.2	26.2	26.2
119	26.4	26.3	26.4	26.4	26.2	26.2	26.1	26.2
120	26.4	26.3	26.4	26.4	26.1	26.2	26.1	26.2
123	26.5	26.5	26.6	26.5	26.2	26.3	26.2	26.3

	Nominal concentration Dexamethasone [µg/L]							
124	26.5	26.4	26.6	26.6	26.3	26.3	26.3	26.5
125	26.5	26.5	26.6	26.6	26.3	26.3	26.2	26.4
126	26.5	26.4	26.4	26.5	26.3	26.3	26.3	26.3
127	26.5	26.4	26.5	26.6	26.3	26.3	26.3	26.4
130	26.6	26.4	26.5	26.4	26.3	26.2	26.3	26.4
131	26.6	26.4	26.5	26.4	26.3	26.3	26.3	26.4
132	26.5	26.3	26.4	26.4	26.2	26.2	26.3	26.4
133	26.5	26.3	26.4	26.3	26.2	26.2	26.3	26.4
134	26.5	26.4	26.5	26.4	26.3	26.2	26.4	26.4
137	26.5	26.4	26.5	26.6	26.4	26.3	26.6	26.5
138	26.4	26.3	26.4	26.5	26.3	26.3	26.5	26.4
139	26.5	26.4	26.4	26.5	26.4	26.4	26.5	26.5
Mean	**26.4**	**26.4**	**26.4**	**26.5**	**26.2**	**26.2**	**26.4**	**26.4**
SD	0.18	0.17	0.24	0.22	0.19	0.20	0.26	0.24
SD%	0.7	0.7	0.9	0.8	0.7	0.8	1.0	0.9
Min	25.9	25.8	25.9	26.0	25.6	25.7	25.9	25.9
Max	26.8	26.8	27.1	27.1	26.7	26.7	27.1	27.1

Table 124: Water temperature [°C]; 1.00 and 3.20 µg Dexamethasone/L

	Nominal concentration Dexamethasone [µg/L]							
	1.00				3.20			
Replicate	A	B	C	D	A	B	C	D
Time of exposure [day]	Water temperature [°C]							
0	25.8	25.9	25.9	26.0	25.9	26.0	26.1	26.3
1	25.9	25.9	26.0	26.0	25.9	26.0	26.1	26.4
4	26.6	26.5	27.0	27.1	26.9	27.0	27.1	27.2
5	26.5	26.5	26.9	27.0	26.9	27.0	27.0	27.1
6	26.3	26.3	26.6	26.7	26.6	26.8	26.8	26.9
7	26.3	26.3	26.7	26.7	26.6	26.7	26.8	26.8
8	26.3	26.3	26.7	26.7	26.6	26.7	26.8	26.8
11	26.4	26.4	26.7	26.8	26.7	26.8	26.9	26.8
12	26.5	26.4	26.7	26.8	26.9	27.0	27.0	27.0
13	26.3	26.3	26.6	26.6	26.6	26.6	26.7	26.7
14	26.3	26.2	26.6	26.6	26.5	26.7	26.6	26.5
15	26.3	26.2	26.6	26.6	26.5	26.6	26.6	26.5
18	26.3	26.3	26.5	26.5	26.6	26.6	26.6	26.6
19	26.2	26.2	26.5	26.5	26.6	26.6	26.6	26.6
20	26.2	26.2	26.5	26.5	26.6	26.7	26.7	26.6
21	26.2	26.2	26.6	26.5	26.6	26.7	26.7	26.6
22	26.2	26.2	26.5	26.6	26.6	26.7	26.6	26.7
25	26.3	26.3	26.6	26.6	26.6	26.6	26.6	26.7
26	26.2	26.1	26.4	26.4	26.5	26.6	26.4	26.4
27	26.2	26.2	26.4	26.4	26.5	26.6	26.4	26.4
28	26.3	26.3	26.6	26.6	26.6	26.7	26.5	26.6
29	26.3	26.3	26.6	26.6	26.6	26.7	26.5	26.6
32	26.2	26.1	26.3	26.3	26.3	26.5	26.3	26.4
33	26.1	26.1	26.4	26.4	26.4	26.6	26.4	26.5
34	26.2	26.2	26.4	26.4	26.4	26.6	26.4	26.5
35	26.0	26.0	26.3	26.3	26.4	26.5	26.3	26.4
36	26.0	26.0	26.3	26.3	26.2	26.4	26.3	26.4
39	26.3	26.3	26.4	26.4	26.5	26.6	26.5	26.4

	Nominal concentration Dexamethasone [µg/L]							
40	26.2	26.2	26.4	26.3	26.5	26.6	26.5	26.4
41	26.2	26.1	26.5	26.3	26.5	26.6	26.4	26.5
42	26.2	26.2	26.5	26.3	26.5	26.6	26.5	26.5
43	26.1	26.0	26.2	26.2	26.4	26.5	26.3	26.3
46	26.2	26.1	26.4	26.3	26.5	26.7	26.4	26.4
47	26.2	26.1	26.4	26.3	26.5	26.6	26.4	26.4
48	26.0	26.0	26.1	26.1	26.3	26.3	26.2	26.3
49	26.1	26.1	26.2	26.2	26.3	26.4	26.4	26.4
50	26.1	26.0	26.2	26.1	26.4	26.6	26.2	26.4
53	26.0	25.8	26.1	26.1	26.4	26.5	26.2	26.3
54	25.9	25.8	26.0	26.0	26.2	26.3	26.1	26.3
55	25.8	25.9	26.2	26.0	26.3	26.4	26.2	26.3
56	26.0	25.9	26.2	26.1	26.2	26.5	26.1	26.4
57	26.0	25.8	26.2	26.1	26.3	26.5	26.2	26.4
60	26.0	25.8	26.1	26.0	26.2	26.4	26.1	26.4
61	25.9	25.8	26.1	26.0	26.1	26.3	26.1	26.2
62	26.0	25.9	26.1	26.0	26.5	26.6	26.3	26.4
63	25.9	25.8	26.1	26.0	26.2	26.5	26.2	26.3
64	26.0	25.8	26.1	26.0	26.1	26.4	26.2	26.2
67	25.9	25.8	26.0	26.0	26.1	26.0	26.2	26.2
68	26.0	25.9	26.1	26.1	26.2	26.4	26.3	26.2
69	26.0	25.9	26.1	26.1	26.3	26.4	26.3	26.3
70	26.0	25.9	26.1	26.1	26.3	26.4	26.3	26.3
71	25.9	25.8	26.1	26.0	26.1	26.3	26.2	26.2
74	25.8	25.7	25.9	25.8	26.0	26.1	25.9	25.9
75	25.9	25.7	25.9	25.9	26.0	26.1	25.9	25.9
76	25.9	25.9	26.1	26.0	25.8	25.8	26.1	26.0
77	25.8	25.8	26.1	26.0	26.0	26.3	26.1	26.2
78	25.7	25.7	26.0	25.9	26.0	26.1	26.0	26.0
81	26.0	26.0	26.0	26.0	26.3	26.4	26.2	26.3
82	26.0	26.0	26.0	26.0	26.4	26.5	26.2	26.3
83	26.0	26.0	26.0	26.0	26.4	26.4	26.2	26.3

	Nominal concentration Dexamethasone [µg/L]							
84	26.2	26.1	26.1	26.1	26.4	26.6	26.3	26.5
85	26.0	26.0	26.1	26.1	26.4	26.5	26.2	26.5
88	26.1	26.0	26.1	26.1	26.5	26.6	26.3	26.5
89	26.0	26.0	26.1	26.1	26.4	26.4	26.2	26.4
90	26.0	26.0	26.1	26.0	26.4	26.4	26.1	26.3
91	26.0	26.0	26.0	26.0	26.4	26.4	26.0	26.2
92	26.0	26.0	26.1	26.0	26.4	26.4	26.1	26.2
95	26.1	26.1	26.1	26.2	26.5	26.5	26.3	26.5
96	26.1	26.1	26.2	26.1	26.5	26.6	26.4	26.4
97	26.1	26.1	26.2	26.1	26.5	26.6	26.4	26.4
98	26.2	26.1	26.1	26.1	26.7	26.8	26.4	26.6
99	26.2	26.2	26.1	26.1	26.6	26.7	26.4	26.5
102	26.3	26.2	26.2	26.2	26.7	26.7	26.4	26.5
103	26.3	26.3	26.3	26.3	26.6	26.7	26.5	26.5
104	26.2	26.1	26.2	26.2	26.5	26.6	26.4	26.4
105	26.1	26.1	26.2	26.2	26.5	26.6	26.4	26.4
106	26.1	26.1	26.2	26.1	26.5	26.6	26.3	26.4
109	26.1	26.1	26.1	26.1	26.5	26.5	26.3	26.3
110	26.1	26.1	26.1	26.1	26.5	26.5	26.3	26.3
111	26.1	26.1	26.1	26.1	26.5	26.5	26.3	26.3
112	26.1	26.1	26.1	26.1	26.4	26.6	26.2	26.3
113	26.2	26.1	26.1	26.0	26.6	26.6	26.2	26.3
116	26.1	26.1	26.1	26.0	26.5	26.5	26.3	26.3
117	26.1	26.1	26.1	26.0	26.5	26.6	26.3	26.3
118	26.1	26.1	26.1	26.1	26.5	26.5	26.3	26.3
119	26.1	26.1	26.0	26.0	26.6	26.6	26.3	26.4
120	26.1	26.1	26.1	26.1	26.5	26.5	26.3	26.3
123	26.2	26.2	26.2	26.2	26.6	26.6	26.4	26.4
124	26.2	26.2	26.2	26.1	26.7	26.7	26.4	26.5
125	26.2	26.2	26.2	26.2	26.6	26.7	26.4	26.5
126	26.2	26.2	26.1	26.1	26.6	26.6	26.3	26.3
127	26.2	26.2	26.1	26.1	26.7	26.7	26.3	26.4

	Nominal concentration Dexamethasone [µg/L]							
130	26.2	26.1	26.2	26.1	26.7	26.7	26.4	26.5
131	26.2	26.2	26.2	26.1	26.7	26.7	26.4	26.5
132	26.2	26.2	26.1	26.1	26.6	26.6	26.4	26.5
133	26.2	26.2	26.2	26.1	26.7	26.6	26.4	26.5
134	26.2	26.2	26.2	26.2	26.7	26.6	26.4	26.5
137	26.3	26.3	26.2	26.2	26.7	26.6	26.4	26.5
138	26.2	26.2	26.2	26.1	26.6	26.5	26.3	26.4
139	26.3	26.3	26.2	26.2	26.6	26.5	26.4	26.5
140	-	-	-	-	26.6	26.6	26.5	26.6
141	-	-	-	-	-	-	-	-
Mean	**26.1**	**26.1**	**26.2**	**26.2**	**26.5**	**26.5**	**26.4**	**26.4**
SD	0.16	0.18	0.23	0.25	0.22	0.20	0.22	0.21
SD%	0.6	0.7	0.9	1.0	0.8	0.8	0.8	0.8
Min	25.7	25.7	25.9	25.8	25.8	25.8	25.9	25.9
Max	26.6	26.5	27.0	27.1	26.9	27.0	27.1	27.2

Table 125: Water temperature [°C]; 10.0, 32.0 and 100 µg Dexamethasone/L

	Nominal concentration Dexamethasone [µg/L]											
	10.0				32.0				100			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Time of exposure [day]	Water temperature [°C]											
0	25.8	25.8	26.3	26.3	25.9	25.9	26.4	26.4	26.2	26.5	26.5	26.8
1	25.9	25.9	26.3	26.3	25.9	25.9	26.4	26.5	26.2	26.5	26.5	26.8
4	26.7	26.7	27.0	27.0	26.8	26.8	27.0	27.1	26.9	26.9	27.2	27.2
5	26.6	26.6	26.8	26.9	26.8	26.8	26.9	27.0	26.9	26.9	27.1	27.2
6	26.3	26.4	26.7	26.7	26.5	26.6	26.7	26.8	26.6	26.6	26.9	26.9
7	26.4	26.5	26.7	26.7	26.5	26.6	26.7	26.8	26.6	26.6	26.9	26.9
8	26.4	26.5	26.7	26.7	26.5	26.6	26.7	26.7	26.6	26.6	26.8	26.8
11	26.4	26.5	26.6	26.6	26.5	26.6	26.7	26.8	26.6	26.7	27.0	27.0
12	26.5	26.7	26.6	26.5	26.5	26.6	26.7	26.8	26.7	26.7	27.0	26.9
13	26.4	26.5	26.5	26.5	26.5	26.6	26.7	26.6	26.7	26.6	26.7	26.9
14	26.4	26.5	26.5	26.5	26.5	26.5	26.6	26.7	26.6	26.7	26.8	26.8
15	26.4	26.4	26.5	26.5	26.5	26.5	26.6	26.6	26.6	26.7	26.7	26.7
18	26.3	26.3	26.5	26.5	26.5	26.5	26.6	26.6	26.6	26.6	26.7	26.8
19	26.3	26.3	26.5	26.5	26.4	26.4	26.6	26.7	26.5	26.5	26.6	26.8
20	26.4	26.4	26.4	26.4	26.5	26.5	26.6	26.7	26.6	26.6	26.7	26.7
21	26.4	26.4	26.5	26.5	26.5	26.5	26.7	26.7	26.6	26.6	26.7	26.8
22	26.3	26.3	26.6	26.6	26.4	26.4	26.6	26.7	26.6	26.6	26.8	26.8
25	26.3	26.4	26.6	26.6	26.4	26.4	26.6	26.6	26.6	26.6	26.8	26.8
26	26.1	26.2	26.5	26.4	26.3	26.3	26.5	26.6	26.5	26.5	26.7	26.7
27	26.1	26.2	26.4	26.4	26.3	26.3	26.5	26.5	26.5	26.5	26.6	26.6
28	26.2	26.4	26.6	26.5	26.4	26.4	26.6	26.6	26.5	26.5	26.8	26.9
29	26.3	26.5	26.6	26.6	26.4	26.4	26.6	26.6	26.6	26.6	26.9	26.9
32	26.1	26.2	26.4	26.3	26.2	26.2	26.3	26.4	26.4	26.3	26.5	26.6
33	26.1	26.2	26.5	26.4	26.3	26.3	26.5	26.5	26.4	26.4	26.6	26.7
34	26.1	26.2	26.5	26.4	26.3	26.3	26.5	26.5	26.4	26.4	26.6	26.6
35	26.0	26.1	26.3	26.3	26.2	26.1	26.4	26.4	26.4	26.3	26.6	26.7
36	26.0	26.0	26.3	26.3	26.1	26.0	26.3	26.4	26.2	26.1	26.5	26.5

	Nominal concentration Dexamethasone [µg/L]											
39	26.3	26.3	26.4	26.4	26.4	26.3	26.5	26.5	26.5	26.5	26.7	26.8
40	26.2	26.3	26.4	26.4	26.3	26.3	26.5	26.5	26.5	26.5	26.7	26.7
41	26.1	26.3	26.4	26.4	26.2	26.2	26.3	26.5	26.5	26.5	26.7	26.8
42	26.2	26.3	26.4	26.4	26.3	26.3	26.4	26.5	26.5	26.5	26.7	26.7
43	26.0	26.1	26.3	26.2	26.1	26.1	26.3	26.4	26.4	26.3	26.6	26.6
46	26.1	26.2	26.4	26.3	26.2	26.2	26.4	26.5	26.4	26.4	26.6	26.7
47	26.1	26.1	26.3	26.3	26.2	26.2	26.4	26.5	26.4	26.4	26.6	26.7
48	26.0	26.1	26.2	26.2	26.1	26.0	26.3	26.3	26.2	26.2	26.5	26.6
49	26.1	26.1	26.3	26.2	26.1	26.1	26.4	26.4	26.2	26.3	26.5	26.5
50	26.1	26.2	26.3	26.2	26.2	26.2	26.4	26.4	26.5	26.4	26.7	26.7
53	26.1	26.1	26.3	26.4	26.1	26.1	26.4	26.5	26.4	26.4	26.7	26.7
54	26.0	26.0	26.2	26.2	26.0	26.0	26.2	26.3	26.3	26.2	26.5	26.5
55	25.8	26.0	26.2	26.2	26.0	26.0	26.2	26.4	26.3	26.3	26.6	26.6
56	26.0	26.1	26.2	26.2	26.2	26.2	26.3	26.5	26.4	26.3	26.6	26.8
57	26.0	26.1	26.3	26.3	26.1	26.1	26.3	26.3	26.4	26.4	26.7	26.6
60	26.1	26.1	26.3	26.2	26.2	26.0	26.4	26.3	26.4	26.4	26.6	26.8
61	26.0	26.1	26.2	26.2	26.0	26.0	26.2	26.3	26.3	26.2	26.5	26.5
62	26.2	26.3	26.4	26.3	26.3	26.3	26.4	26.5	26.6	26.5	26.7	26.9
63	26.1	26.1	26.2	26.2	26.2	26.1	26.3	26.4	26.4	26.3	26.7	26.8
64	26.1	26.1	26.2	26.1	26.2	26.1	26.3	26.3	26.3	26.2	26.5	26.6
67	26.0	25.9	26.1	26.2	26.1	26.1	26.2	26.3	26.3	26.2	26.5	26.5
68	26.1	26.1	26.2	26.2	26.2	26.2	26.3	26.3	26.4	26.5	26.6	26.6
69	26.1	26.2	26.2	26.2	26.3	26.2	26.4	26.4	26.5	26.5	26.6	26.7
70	26.1	26.2	26.2	26.2	26.3	26.2	26.4	26.4	26.4	26.5	26.6	26.6
71	26.0	26.0	26.1	26.0	26.1	26.1	26.1	26.1	26.3	26.2	26.4	26.5
74	25.9	25.9	26.0	25.9	26.1	26.0	26.0	26.1	26.3	26.2	26.4	26.4
75	25.9	25.9	26.0	25.9	26.1	26.0	26.0	26.1	26.3	26.2	26.4	26.4
76	26.0	26.1	26.1	26.1	26.3	26.2	26.2	26.3	26.5	26.4	26.6	26.8
77	26.0	26.0	26.0	26.1	26.3	26.2	26.1	26.3	26.6	26.4	26.7	26.8
78	25.8	26.0	25.9	26.0	26.2	26.1	26.0	26.1	26.4	26.2	26.5	26.6
81	26.2	26.2	26.2	26.3	26.5	26.4	26.5	26.5	26.8	26.6	26.9	26.9
82	26.2	26.3	26.2	26.3	26.5	26.4	26.3	26.5	26.6	26.6	26.7	26.9

	Nominal concentration Dexamethasone [µg/L]											
83	26.2	26.3	26.2	26.3	26.5	26.4	26.3	26.5	26.6	26.6	26.7	26.8
84	26.2	26.4	26.4	26.4	26.6	26.6	26.4	26.5	26.8	26.7	26.8	26.9
85	26.3	26.4	26.4	26.4	26.6	26.6	26.5	26.5	26.8	26.8	26.8	27.0
88	26.3	26.4	26.4	26.5	26.6	26.6	26.6	26.7	26.8	26.7	26.8	26.9
89	26.1	26.3	26.4	26.4	26.4	26.4	26.4	26.4	26.8	26.7	26.7	26.8
90	26.1	26.3	26.4	26.4	26.5	26.5	26.5	26.5	26.8	26.7	26.7	26.8
91	26.1	26.3	26.4	26.3	26.4	26.4	26.4	26.4	26.7	26.7	26.7	26.7
92	26.1	26.3	26.4	26.4	26.5	26.5	26.5	26.5	26.8	26.7	26.7	26.8
95	26.3	26.4	26.5	26.5	26.6	26.6	26.6	26.7	26.9	26.8	26.8	26.9
96	26.2	26.3	26.5	26.4	26.4	26.4	26.4	26.4	26.6	26.6	26.6	26.7
97	26.3	26.3	26.5	26.4	26.5	26.5	26.5	26.5	26.6	26.6	26.6	26.7
98	26.4	26.6	26.6	26.6	26.7	26.7	26.6	26.7	26.8	26.8	26.9	26.9
99	26.4	26.5	26.5	26.5	26.6	26.6	26.6	26.6	26.8	26.8	26.8	26.8
102	26.3	26.4	26.6	26.6	26.6	26.7	26.5	26.7	26.8	26.8	26.8	26.9
103	26.3	26.4	26.6	26.6	26.6	26.7	26.6	26.7	26.7	26.7	26.8	26.9
104	26.2	26.3	26.5	26.4	26.4	26.5	26.4	26.4	26.7	26.6	26.7	26.7
105	26.2	26.2	26.4	26.4	26.4	26.4	26.3	26.3	26.7	26.7	26.7	26.7
106	26.2	26.2	26.4	26.4	26.5	26.4	26.3	26.4	26.7	26.7	26.7	26.7
109	26.2	26.2	26.3	26.3	26.4	26.4	26.2	26.4	26.6	26.6	26.6	26.7
110	26.1	26.3	26.4	26.4	26.4	26.4	26.2	26.4	26.6	26.6	26.6	26.8
111	26.2	26.3	26.4	26.4	26.4	26.4	26.2	26.4	26.6	26.6	26.6	26.7
112	26.2	26.3	26.3	26.3	26.6	26.5	26.3	26.3	26.8	26.7	26.7	26.8
113	26.2	26.3	26.3	26.3	26.6	26.5	26.2	26.3	26.8	26.7	26.7	26.8
116	26.2	26.3	26.2	26.2	26.4	26.4	26.2	26.2	26.7	26.7	26.7	26.8
117	26.1	26.3	26.2	26.2	26.4	26.5	26.2	26.2	26.7	26.7	26.7	26.8
118	26.2	26.3	26.2	26.2	26.4	26.4	26.2	26.2	26.7	26.7	26.7	26.8
119	26.2	26.3	26.2	26.2	26.5	26.5	26.1	26.2	26.7	26.7	26.7	26.7
120	26.2	26.3	26.2	26.2	26.5	26.5	26.2	26.2	26.7	26.7	26.7	26.7
123	26.3	26.4	26.3	26.3	26.5	26.5	26.3	26.3	26.7	26.7	26.8	26.8
124	26.3	26.5	26.3	26.4	26.6	26.7	26.3	26.5	26.8	26.8	26.8	26.9
125	26.3	26.5	26.3	26.4	26.6	26.6	26.3	26.4	26.7	26.7	26.7	26.8
126	26.2	26.3	26.4	26.4	26.6	26.6	26.3	26.3	26.7	26.7	26.7	26.7

	Nominal concentration Dexamethasone [µg/L]											
127	26.2	26.3	26.3	26.3	26.6	26.6	26.3	26.3	26.8	26.7	26.7	26.8
130	26.3	26.3	26.3	26.3	26.5	26.5	26.2	26.2	26.8	26.7	26.7	26.8
131	26.3	26.3	26.3	26.3	26.6	26.6	26.3	26.3	26.8	26.7	26.7	26.8
132	26.3	26.3	26.3	26.3	26.5	26.5	26.3	26.2	26.7	26.6	26.6	26.7
133	26.3	26.4	26.3	26.3	26.6	26.5	26.3	26.3	26.7	26.7	26.7	26.8
134	26.3	26.4	26.3	26.3	26.6	26.5	26.3	26.3	26.7	26.7	26.7	26.7
137	26.3	26.4	26.5	26.4	26.6	26.6	26.3	26.4	27.0	27.0	26.9	26.9
138	26.3	26.3	26.4	26.3	26.5	26.5	26.3	26.3	26.8	26.8	26.7	26.7
139	26.4	26.4	26.4	26.3	26.6	26.6	26.4	26.4	26.7	26.7	26.7	26.7
140	26.5	26.5	26.4	26.4	26.7	26.7	26.5	26.6	26.8	26.8	26.8	26.8
141	-	-	-	-	-	26.8	26.7	26.8	27.0	27.0	26.9	26.9
Mean	**26.2**	**26.3**	**26.4**	**26.4**	**26.4**	**26.4**	**26.4**	**26.5**	**26.6**	**26.6**	**26.7**	**26.8**
SD	0.16	0.18	0.18	0.18	0.20	0.22	0.19	0.19	0.19	0.19	0.14	0.14
SD%	0.6	0.7	0.7	0.7	0.7	0.8	0.7	0.7	0.7	0.7	0.5	0.5
Min	25.8	25.8	25.9	25.9	25.9	25.9	26.0	26.1	26.2	26.1	26.4	26.4
Max	26.7	26.7	27.0	27.0	26.8	26.8	27.0	27.1	27.0	27.0	27.2	27.2

C.2.2 Test conditions: oxygen saturation

Table 126: O_2 saturation [% ASV]; control and 0.32 µg Dexamethasone/L

	Nominal concentration Dexamethasone [µg/L]							
	control				0.32			
Replicate	A	B	C	D	A	B	C	D
Time of exposure [day]	O_2 saturation [% ASV]							
0	109	110	109	110	108	108	111	109
4	104	104	102	101	101	101	101	99
7	102	100	100	100	101	101	101	100
11	100	100	100	100	99	99	99	99
14	99	99	98	99	99	99	98	97
18	100	100	100	100	99	99	98	98
21	98	100	100	101	101	101	100	100
27	100	100	100	100	99	99	101	101
29	93	97	97	97	98	98	98	97
32	97	100	101	101	103	103	102	102
35	105	105	105	105	105	106	107	106
39	102	102	102	102	100	102	102	102
42	100	102	102	102	103	104	104	104
46	97	99	100	100	101	103	103	103
49	99	103	104	104	105	105	105	105
53	96	103	101	102	103	102	101	98
56	96	96	95	94	96	97	96	93
60	88	91	92	93	95	96	96	95
63	108	108	107	107	107	110	109	108
67	109	113	110	109	110	110	112	111
71	109	113	113	113	114	114	114	115
74	110	110	111	110	111	111	111	110
77	104	102	101	101	102	103	102	102
81	112	115	113	112	112	113	113	113
84	111	114	112	112	112	112	113	113
88	105	107	108	108	108	108	109	108

	Nominal concentration Dexamethasone [µg/L]							
91	107	111	111	109	109	110	109	108
95	106	108	109	108	107	107	107	106
98	106	107	107	107	105	106	108	109
102	104	106	103	102	103	103	103	103
105	106	105	106	106	104	105	105	104
109	104	110	104	104	105	105	105	105
112	104	110	104	105	104	103	104	105
116	107	112	111	109	109	110	109	108
119	105	113	107	107	108	107	106	106
123	105	114	107	107	108	109	109	108
126	106	113	106	107	106	107	107	105
130	100	113	102	104	106	107	106	105
133	106	110	106	107	106	107	107	108
137	106	111	105	106	106	107	105	105
139	105	111	105	106	106	105	104	105
Mean	**103**	**106**	**104**	**104**	**104**	**105**	**105**	**104**
SD	5	6	5	5	4	5	5	5
RSD	4.9	5.7	4.7	4.5	4.3	4.3	4.5	4.8
Min	88	91	92	93	95	96	96	93
Max	112	115	113	113	114	114	114	115

Table 127: O_2 saturation [% ASV]; 1.00 and 3.20 µg Dexamethasone/L

	Nominal concentration Dexamethasone [µg/L]							
	1.00				3.20			
Replicate	A	B	C	D	A	B	C	D
Time of exposure [day]	O_2 saturation [% ASV]							
0	108	108	108	107	106	106	106	106
4	99	100	99	100	99	99	99	99
7	100	100	100	100	99	99	99	98
11	98	98	98	97	97	97	99	99
14	97	96	96	99	99	97	95	95
18	99	100	99	99	98	98	100	100
21	100	100	100	100	99	98	98	99
27	100	100	99	100	99	99	98	98
29	97	98	98	97	97	96	96	96
32	101	102	102	101	101	100	100	99
35	105	105	105	105	105	103	102	103
39	100	101	101	102	101	100	100	101
42	102	102	103	102	102	102	101	101
46	102	102	101	100	99	98	97	97
49	105	104	103	103	103	102	101	101
53	101	101	97	96	97	96	97	96
56	95	95	95	95	94	95	95	94
60	96	94	94	95	95	95	95	95
63	107	107	106	106	107	107	106	106
67	110	110	110	109	109	109	109	108
71	114	114	114	113	113	112	112	111
74	110	109	109	107	108	108	110	110
77	102	102	102	101	101	101	100	100
81	112	112	111	110	110	110	110	110
84	112	112	112	110	111	111	110	110
88	108	107	107	106	106	103	105	105
91	108	108	107	107	107	107	107	106
95	106	106	105	105	105	105	104	104

	Nominal concentration Dexamethasone [µg/L]							
98	106	107	107	108	109	109	108	109
102	103	103	103	102	103	102	101	101
105	104	105	106	106	105	105	103	103
109	106	105	105	104	104	104	103	103
112	105	105	105	105	106	106	104	105
116	108	108	108	107	107	106	105	105
119	107	107	108	107	106	106	107	107
123	108	108	107	106	106	106	105	105
126	105	107	107	107	106	106	106	106
130	106	106	104	103	104	104	102	102
133	107	106	106	107	108	107	107	108
137	104	104	105	104	105	106	106	106
139	105	105	105	105	106	106	105	104
Mean	**104**	**104**	**104**	**103**	**103**	**103**	**103**	**103**
SD	5	5	5	4	5	5	5	5
RSD	4.4	4.4	4.5	4.2	4.4	4.5	4.5	4.5
Min	95	94	94	95	94	95	95	94
Max	114	114	114	113	113	112	112	111

Table 128: O_2 saturation [% ASV]; 10.0, 32.0 and 100 µg Dexamethasone/L

	Nominal concentration Dexamethasone [µg/L]											
	10.0				32.0				100			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Time of exposure [day]	O_2 saturation [% ASV]											
0	106	106	106	106	105	105	105	105	105	105	105	105
4	98	98	99	98	98	98	98	97	97	97	97	97
7	99	99	99	99	98	98	98	98	97	97	97	96
11	99	99	98	98	99	99	97	97	97	97	97	97
14	96	96	96	95	94	94	95	95	95	96	96	96
18	100	100	99	99	98	97	97	96	98	98	97	98
21	99	100	100	99	99	100	99	98	97	96	96	94
27	100	100	99	99	97	97	97	97	99	98	98	98
29	97	97	97	96	97	98	97	97	96	96	96	95
32	100	100	100	99	99	100	100	98	98	98	98	96
35	104	104	103	102	102	104	104	103	103	102	102	101
39	102	102	98	98	100	99	101	103	101	101	102	101
42	101	102	102	101	101	102	102	101	101	101	101	99
46	98	98	98	96	97	98	98	97	97	97	97	95
49	101	101	101	101	101	102	102	102	102	101	100	98
53	97	97	96	92	96	100	96	94	95	95	94	94
56	95	95	95	94	94	95	96	95	95	94	94	93
60	95	96	96	95	95	96	95	96	95	95	95	95
63	107	107	107	105	105	110	109	107	107	107	106	105
67	108	108	108	106	107	109	109	109	109	108	108	106
71	111	112	112	110	109	111	111	111	111	110	110	110
74	109	109	111	110	111	111	111	111	112	112	111	111
77	101	101	101	100	101	102	102	102	101	100	100	98
81	110	110	110	109	109	111	111	110	110	109	108	107
84	110	110	110	109	109	111	111	111	112	110	109	109
88	105	106	106	105	106	106	106	105	105	104	103	102
91	107	107	107	106	107	107	107	107	107	107	106	105

	Nominal concentration Dexamethasone [µg/L]											
95	104	105	104	104	105	105	105	105	105	104	103	103
98	109	109	107	107	108	107	106	106	106	106	106	106
102	102	102	101	101	102	102	102	101	102	101	100	100
105	104	103	105	105	105	105	105	105	106	106	106	106
109	103	104	103	102	103	104	104	103	104	103	101	101
112	105	106	104	104	103	105	105	105	104	103	103	103
116	105	105	105	104	105	105	105	104	104	104	102	101
119	107	107	106	105	106	105	107	107	105	105	105	105
123	106	107	106	105	106	106	106	105	106	104	103	101
126	107	108	108	107	106	106	107	106	108	104	104	105
130	103	104	102	102	102	103	102	101	101	99	98	97
133	106	106	106	106	107	107	106	107	106	105	105	106
137	107	107	106	106	105	104	105	105	107	107	106	106
139	104	103	104	105	104	105	106	105	105	105	105	105
Mean	**103**	**103**	**103**	**102**	**102**	**103**	**103**	**103**	**103**	**102**	**102**	**101**
SD	4	4	5	5	5	5	5	5	5	5	5	5
RSD	4.3	4.3	4.4	4.5	4.4	4.5	4.7	4.8	4.9	4.7	4.6	4.8
Min	95	95	95	92	94	94	95	94	95	94	94	93
Max	111	112	112	110	111	111	111	111	112	112	111	111

C.2.3 Test conditions: pH values

Table 129: pH; control and 0.32 µg Dexamathasone/L

	Nominal concentration Dexamethasone [µg/L]							
	Control				0.32			
Replicate	A	B	C	D	A	B	C	D
Time of exposure [day]	pH value							
0	7.95	7.97	8.00	8.00	7.93	7.90	7.94	7.97
4	7.89	7.98	8.20	8.18	8.17	8.17	8.19	8.16
7	7.88	7.88	7.96	7.94	7.93	7.90	7.95	7.94
11	7.90	7.92	7.93	7.93	7.95	7.95	7.98	7.97
14	8.00	8.00	7.98	8.00	8.02	8.03	8.02	8.03
18	7.90	7.92	7.94	7.95	7.95	7.95	8.00	7.98
21	8.06	8.05	8.08	8.10	8.09	8.06	8.08	8.10
27	8.08	8.10	8.11	8.11	8.15	8.17	8.13	8.15
29	7.73	7.77	7.82	7.87	7.86	7.85	7.84	7.86
32	7.77	7.77	7.80	7.85	7.87	7.84	7.84	7.87
35	8.01	8.00	8.01	8.05	8.08	8.08	8.06	8.07
39	8.01	8.03	8.05	8.05	8.03	8.03	8.05	8.05
42	7.83	7.86	7.91	7.93	7.93	7.94	7.96	7.93
46	7.75	7.77	7.81	7.85	7.88	7.88	7.90	7.90
49	7.85	7.93	7.94	8.00	8.01	8.01	8.04	8.00
53	8.07	8.05	8.05	8.11	8.17	8.12	8.17	8.12
56	7.89	7.87	7.88	7.87	7.95	7.94	7.90	7.88
60	7.92	8.02	7.99	7.99	8.04	8.05	8.05	8.00
63	8.00	7.85	7.79	7.87	8.00	7.96	7.99	7.94
67	7.95	8.08	8.02	8.07	8.16	8.16	8.20	8.16
71	7.86	7.94	7.93	7.95	8.01	8.00	8.06	8.04
74	7.92	7.95	8.00	8.00	8.01	8.02	8.00	8.02
77	7.96	8.08	8.01	8.08	8.14	8.12	8.17	8.12
81	8.01	8.03	8.02	8.06	8.13	8.11	8.18	8.10
84	7.90	8.06	7.98	8.01	8.04	8.04	8.17	8.08
88	7.98	8.04	8.01	8.05	8.05	8.07	8.10	8.07

	Nominal concentration Dexamethasone [µg/L]							
91	7.86	8.01	7.95	7.96	7.95	7.98	7.99	7.97
95	8.02	8.16	8.08	8.11	8.14	8.17	8.16	8.15
98	8.01	8.05	8.07	8.07	8.15	8.15	8.17	8.18
102	8.11	8.30	8.14	8.17	8.22	8.22	8.22	8.22
105	8.10	8.35	8.11	8.15	8.17	8.14	8.20	8.20
109	8.08	8.37	8.13	8.20	8.27	8.28	8.27	8.25
112	8.05	8.35	8.12	8.12	8.20	8.22	8.20	8.22
116	8.02	8.28	8.02	8.09	8.13	8.11	8.11	8.11
119	7.97	8.30	8.01	8.02	8.03	8.00	8.01	8.01
123	7.95	8.28	7.94	8.01	8.05	8.06	8.08	8.07
126	8.00	8.31	8.05	8.05	8.02	8.05	8.00	8.01
130	7.83	8.20	7.81	7.94	7.95	7.97	7.96	7.92
133	8.02	8.25	8.01	8.03	8.05	8.05	8.03	8.05
137	7.99	8.27	8.02	8.05	8.00	7.99	8.02	8.02
139	8.02	8.35	8.05	8.05	8.01	8.00	8.01	8.00
Mean	**7.94**	**8.03**	**7.98**	**8.01**	**8.03**	**8.03**	**8.05**	**8.03**
SD	7.73	7.77	7.79	7.85	7.86	7.84	7.84	7.86
RSD	8.11	8.37	8.20	8.20	8.27	8.28	8.27	8.25
Min	0.09	0.17	0.10	0.09	0.10	0.10	0.11	0.10
Max	1.2	2.2	1.2	1.1	1.2	1.3	1.3	1.3

Table 130: pH; 1.00 and 3.20 µg Dexamathasone/L

	Nominal concentration Dexamethasone [µg/L]							
	1.00				3.20			
Replicate	A	B	C	D	A	B	C	D
Time of exposure [day]	pH value							
0	7.99	8.00	8.02	8.01	8.05	8.07	8.08	8.08
4	8.17	8.17	8.19	8.20	8.18	8.17	8.23	8.22
7	7.93	7.95	7.98	7.98	7.95	7.93	7.99	7.99
11	7.93	7.95	8.00	8.00	7.98	7.97	8.00	8.00
14	8.00	8.00	7.95	8.00	7.98	8.00	8.05	8.03
18	8.00	8.00	8.02	8.00	8.02	8.03	8.00	7.99
21	8.07	8.07	8.11	8.13	8.11	8.10	8.12	8.19
27	8.15	8.13	8.08	8.08	8.10	8.11	8.15	8.17
29	7.87	7.88	7.89	7.89	7.87	7.88	7.92	7.93
32	7.89	7.90	7.91	7.90	7.89	7.87	7.90	7.90
35	8.08	8.06	8.05	8.01	8.03	8.01	8.03	8.04
39	8.06	8.07	8.04	8.04	8.06	8.06	8.10	8.11
42	7.97	7.98	7.98	7.97	7.96	7.96	7.97	7.98
46	7.89	7.89	7.91	7.93	7.91	7.93	7.91	7.92
49	7.99	7.99	7.98	7.99	7.97	7.96	7.97	7.98
53	8.15	8.12	8.00	8.12	8.09	8.10	8.12	8.15
56	7.94	7.92	7.93	7.95	7.93	7.93	7.96	7.96
60	8.04	8.06	8.06	8.03	8.02	8.02	8.02	8.01
63	7.96	7.94	7.95	7.91	8.04	7.97	8.02	8.06
67	8.16	8.12	8.10	8.11	8.18	8.17	8.17	8.16
71	8.02	8.01	8.02	8.02	8.04	8.04	8.05	8.05
74	8.05	8.06	8.06	8.06	8.04	8.03	8.04	8.05
77	8.12	8.08	8.10	8.11	8.12	8.08	8.10	8.13
81	8.12	8.09	8.08	8.10	8.14	8.14	8.14	8.15
84	8.07	8.06	8.07	8.08	8.12	8.13	8.11	8.12
88	8.07	8.06	8.08	8.10	8.12	8.16	8.13	8.11
91	7.98	7.98	7.99	7.98	8.00	8.00	8.00	8.01
95	8.13	8.09	8.12	8.10	8.14	8.14	8.11	8.11

	Nominal concentration Dexamethasone [µg/L]							
98	8.10	8.13	8.16	8.16	8.10	8.11	8.15	8.16
102	8.21	8.19	8.19	8.18	8.19	8.20	8.18	8.18
105	8.20	8.21	8.23	8.21	8.21	8.20	8.24	8.25
109	8.24	8.22	8.20	8.20	8.24	8.24	8.21	8.22
112	8.25	8.25	8.26	8.28	8.25	8.23	8.24	8.24
116	8.13	8.10	8.08	8.07	8.10	8.11	8.08	8.10
119	8.04	8.05	8.03	8.00	8.00	8.01	8.05	8.06
123	8.06	8.04	8.01	8.00	8.00	8.01	8.01	8.04
126	8.00	8.02	8.02	8.02	8.04	8.00	8.05	8.06
130	7.96	7.94	7.92	7.94	7.94	7.92	7.95	7.97
133	8.01	8.02	8.02	8.04	8.04	8.04	8.08	8.10
137	8.04	8.05	8.06	8.05	8.07	8.04	7.99	8.00
139	8.05	8.07	8.10	8.09	8.08	8.09	8.12	8.11
Mean	**8.04**	**8.04**	**8.04**	**8.04**	**8.05**	**8.04**	**8.06**	**8.07**
SD	7.87	7.88	7.89	7.89	7.87	7.87	7.90	7.90
RSD	8.25	8.25	8.26	8.28	8.25	8.24	8.24	8.25
Min	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Max	1.2	1.1	1.1	1.1	1.1	1.2	1.1	1.1

Table 131: pH; 10.0 and 32.0 and 100 µg Dexamathasone/L

	Nominal concentration Dexamethasone [µg/L]											
	10.0				32.0				100			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Time of exposure [day]	pH value											
0	8.06	8.06	8.08	8.08	8.07	8.07	8.09	8.09	8.10	8.11	8.12	8.12
4	8.21	8.21	8.23	8.22	8.21	8.20	8.24	8.22	8.18	8.17	8.21	8.23
7	7.98	7.97	7.96	7.98	7.99	7.99	8.00	8.02	8.02	8.01	8.03	8.03
11	7.93	7.95	7.96	7.95	7.95	7.97	7.98	7.99	8.00	8.00	8.00	8.00
14	8.03	8.05	8.00	8.00	8.00	8.00	8.01	8.00	8.05	8.04	8.05	8.06
18	8.00	7.99	8.05	8.05	7.97	7.98	7.98	8.00	8.02	8.02	8.01	8.02
21	8.21	8.27	8.28	8.19	8.18	8.23	8.17	8.08	7.95	7.85	7.83	7.72
27	8.17	8.17	8.12	8.14	8.15	8.15	8.11	8.11	8.15	8.16	8.17	8.18
29	7.91	7.90	7.92	7.93	7.92	7.93	7.94	7.95	7.96	7.95	7.95	7.93
32	7.90	7.90	7.90	7.88	7.92	7.93	7.93	7.94	7.93	7.92	7.98	7.94
35	8.06	8.02	8.03	8.02	8.05	8.10	8.06	8.07	8.09	8.05	8.06	8.05
39	8.08	8.07	8.05	8.05	8.08	8.07	8.06	8.08	8.10	8.10	8.10	8.10
42	7.98	7.97	7.99	7.99	7.98	7.99	7.99	8.02	8.02	8.01	8.04	8.03
46	7.90	7.90	7.94	7.91	7.93	7.97	7.97	7.98	7.97	7.96	7.98	7.98
49	7.96	7.97	8.01	7.98	7.99	8.04	8.02	8.05	8.03	8.01	8.02	8.01
53	8.07	8.07	8.13	8.08	8.14	8.22	8.14	8.12	8.11	8.12	8.15	8.12
56	7.96	7.94	7.93	7.94	7.95	7.97	7.98	7.95	7.97	7.96	7.97	7.97
60	8.02	8.05	8.05	8.01	8.06	8.08	8.06	8.05	8.05	8.04	8.04	8.05
63	8.02	8.02	8.02	7.96	8.07	8.09	8.02	8.05	8.09	8.08	8.09	8.08
67	8.16	8.14	8.12	8.09	8.15	8.24	8.22	8.23	8.23	8.21	8.23	8.18
71	8.02	8.02	8.02	8.00	8.06	8.10	8.10	8.12	8.11	8.09	8.13	8.09
74	8.05	8.05	8.05	8.05	8.06	8.07	8.06	8.07	8.04	8.04	8.05	8.05
77	8.10	8.09	8.10	8.11	8.12	8.19	8.16	8.21	8.18	8.13	8.20	8.13
81	8.13	8.15	8.14	8.14	8.19	8.23	8.23	8.23	8.25	8.19	8.23	8.17
84	8.09	8.11	8.13	8.09	8.16	8.25	8.19	8.19	8.22	8.17	8.21	8.16
88	8.10	8.09	8.10	8.12	8.12	8.15	8.14	8.16	8.15	8.14	8.18	8.15
91	7.98	7.96	7.98	7.99	8.01	8.04	8.05	8.05	8.06	8.02	8.08	8.05

	Nominal concentration Dexamethasone [µg/L]											
95	8.10	8.08	8.10	8.13	8.16	8.20	8.17	8.21	8.20	8.16	8.19	8.16
98	8.15	8.16	8.11	8.11	8.13	8.14	8.10	8.12	8.15	8.14	8.15	8.15
102	8.17	8.17	8.17	8.18	8.21	8.23	8.23	8.25	8.26	8.22	8.24	8.22
105	8.26	8.26	8.24	8.25	8.27	8.28	8.28	8.25	8.25	8.25	8.24	8.23
109	8.19	8.18	8.19	8.20	8.23	8.27	8.26	8.28	8.28	8.24	8.24	8.23
112	8.26	8.28	8.25	8.25	8.20	8.21	8.19	8.21	8.20	8.22	8.22	8.22
116	8.07	8.06	8.07	8.10	8.12	8.14	8.12	8.17	8.14	8.08	8.08	8.06
119	8.05	8.07	8.01	8.01	8.01	8.00	7.99	8.02	8.03	8.01	8.02	8.01
123	8.02	8.00	8.02	8.01	8.05	8.06	8.07	8.10	8.08	8.03	8.01	8.01
126	8.02	8.06	8.03	8.03	8.10	8.08	8.11	8.10	8.05	8.04	8.05	8.04
130	7.97	7.96	7.96	7.97	7.97	8.00	7.98	8.02	7.99	7.91	7.92	7.94
133	8.05	8.05	8.02	8.02	8.03	8.04	8.05	8.06	8.00	8.02	8.00	8.02
137	8.00	8.00	8.02	8.03	8.02	8.01	7.98	8.00	8.03	8.05	8.02	8.02
139	8.05	8.00	8.02	8.03	8.05	8.04	8.05	8.05	8.00	7.99	8.01	8.03
Mean	**8.05**	**8.05**	**8.05**	**8.05**	**8.06**	**8.08**	**8.07**	**8.08**	**8.08**	**8.06**	**8.07**	**8.06**
SD	7.90	7.90	7.90	7.88	7.92	7.93	7.93	7.94	7.93	7.85	7.83	7.72
RSD	8.26	8.28	8.28	8.25	8.27	8.28	8.28	8.28	8.28	8.25	8.24	8.23
Min	0.09	0.10	0.09	0.09	0.09	0.10	0.09	0.09	0.09	0.09	0.10	0.10
Max	1.1	1.2	1.1	1.1	1.1	1.3	1.2	1.1	1.2	1.2	1.2	1.3

C.2.4 Parenteral generation (F_0), reproduction: fecundity and fertility

Table 132: F_0 generation, reproduction: fecundity and fertilisation rate; control

	Control																			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Time of exposure [day]	Fertilised eggs [n]				Total egg number [n]				Fertilisation rate [%]				Female number [n]				Total egg number per female [n]			
1	372	409	351	434	389	435	381	603	95.6	94.0	92.1	72.0	5	5	5	5	78	87	76	121
2	117	289	232	287	120	298	242	293	97.5	97.0	95.9	98.0	5	5	5	5	24	60	48	59
3	529	415	517	477	588	451	551	547	90.0	92.0	93.8	87.2	5	5	5	5	118	90	110	109
4	529	404	280	471	558	421	330	522	94.8	96.0	84.8	90.2	5	5	5	5	112	84	66	104
5	612	478	414	345	646	499	460	361	94.7	95.8	90.0	95.6	5	5	5	5	129	100	92	72
6	173	270	191	414	189	285	211	439	91.5	94.7	90.5	94.3	5	5	5	5	38	57	42	88
7	489	415	450	400	513	444	485	420	95.3	93.5	92.8	95.2	5	5	5	5	103	89	97	84
8	472	351	114	415	503	373	126	469	93.8	94.1	90.5	88.5	5	5	5	5	101	75	25	94
9	332	290	406	237	359	300	457	257	92.5	96.7	88.8	92.2	5	5	5	5	72	60	91	51
10	182	356	59	370	230	384	78	407	79.1	92.7	75.6	90.9	5	5	5	5	46	77	16	81
11	404	153	410	264	438	164	493	272	92.2	93.3	83.2	97.1	5	5	5	5	88	33	99	54
12	502	411	330	421	535	443	364	437	93.8	92.8	90.7	96.3	5	5	5	5	107	89	73	87
13	172	181	330	250	180	192	392	268	95.6	94.3	84.2	93.3	5	5	5	5	36	38	78	54
14	482	363	485	422	523	367	525	449	92.2	98.9	92.4	94.0	5	5	5	5	105	73	105	90

	Control																			
15	546	257	84	252	657	293	102	293	83.1	87.7	82.4	86.0	5	5	5	5	131	59	20	59
16	298	353	330	399	342	390	400	478	87.1	90.5	82.5	83.5	5	5	5	5	68	78	80	96
17	414	255	208	227	537	284	245	238	77.1	89.8	84.9	95.4	5	5	5	5	107	57	49	48
18	224	352	156	321	246	372	193	353	91.1	94.6	80.8	90.9	5	5	5	5	49	74	39	71
19	43	398	176	494	51	409	202	529	84.3	97.3	87.1	93.4	5	5	5	5	10	82	40	106
20	628	192	216	425	661	195	222	457	95.0	98.5	97.3	93.0	5	5	5	5	132	39	44	91
Mean	**-**	**-**			**413**	**350**	**323**	**405**	**90.8**	**94.2**	**88.0**	**91.3**	**-**	**-**	**-**	**-**	**83**	**70**	**65**	**81**
SD	-	-	-	-	189	95	148	108	5.7	2.9	5.6	5.9	-	-	-	-	38	19	30	22
RSD	-	-	-	-	45.8	27.1	45.8	26.6	6.3	3.0	6.3	6.5	-	-	-	-	45.8	27.1	45.8	26.6
Cumulative egg number [n]					**8265**	**6999**	**6459**	**8092**												

Table 133: F_0 generation, reproduction: fecundity and fertilisation rate; 0.32 µg Dexamethasone/L (nominal), respective 0.33 µg Dexamethasone/L (mean measured)

	Nominal concentration: 0.32 µg Dexamethasone/L Mean measured concentration: 0.33 µg Dexamethasone/L																			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Time of exposure [day]	**Fertilised eggs [n]**				**Total egg number [n]**				**Fertilisation rate [%]**				**Female number [n]**				**Total egg number per female [n]**			
1	317	116	240	241	329	118	245	256	96.4	98.3	98.0	94.1	5	5	5	5	66	24	49	51
2	238	228	219	238	276	240	235	256	86.2	95.0	93.2	93.0	5	5	5	5	55	48	47	51
3	351	415	432	440	388	497	468	505	90.5	83.5	92.3	87.1	5	5	5	5	78	99	94	101
4	257	315	347	409	315	339	351	455	81.6	92.9	98.9	89.9	5	5	5	5	63	68	70	91
5	274	268	212	324	288	316	220	375	95.1	84.8	96.4	86.4	5	5	5	5	58	63	44	75
6	293	251	227	146	314	262	261	175	93.3	95.8	87.0	83.4	5	5	5	5	63	52	52	35
7	248	311	221	309	277	328	234	356	89.5	94.8	94.4	86.8	5	5	5	5	55	66	47	71
8	235	197	118	296	248	203	134	320	94.8	97.0	88.1	92.5	5	5	5	5	50	41	27	64
9	226	234	338	316	311	248	360	345	72.7	94.4	93.9	91.6	5	5	5	5	62	50	72	69
10	210	129	116	205	273	164	136	237	76.9	78.7	85.3	86.5	5	5	5	5	55	33	27	47
11	108	288	201	328	138	327	209	372	78.3	88.1	96.2	88.2	5	5	5	5	28	65	42	74
12	300	73	195	201	375	81	203	221	80.0	90.1	96.1	91.0	5	5	5	5	75	16	41	44
13	380	406	267	179	413	417	312	205	92.0	97.4	85.6	87.3	5	5	5	5	83	83	62	41
14	250	228	230	410	294	236	277	450	85.0	96.6	83.0	91.1	5	5	5	5	59	47	55	90
15	275	311	268	215	423	354	282	270	65.0	87.9	95.0	79.6	5	5	5	5	85	71	56	54

	Nominal concentration: 0.32 µg Dexamethasone/L **Mean measured concentration: 0.33 µg Dexamethasone/L**																			
16	234	166	206	310	350	206	224	377	66.9	80.6	92.0	82.2	5	5	5	5	70	41	45	75
17	266	145	197	172	368	246	256	265	72.3	58.9	77.0	64.9	5	5	5	5	74	49	51	53
18	303	180	175	146	338	208	201	183	89.6	86.5	87.1	79.8	5	5	5	5	68	42	40	37
19	208	193	123	287	247	230	139	336	84.2	83.9	88.5	85.4	5	5	5	5	49	46	28	67
20	324	252	198	119	401	305	250	135	80.8	82.6	79.2	88.1	5	5	5	5	80	61	50	27
Mean	**-**	**-**			**318**	**266**	**250**	**305**	**83.6**	**88.4**	**90.3**	**86.5**	**-**	**-**	**-**	**-**	**64**	**53**	**50**	**61**
SD	-	-	-	-	68	98	80	101	9.4	9.3	6.2	6.5	-	-	-	-	14	20	16	20
RSD	-	-	-	-	21.4	36.7	32.2	33.1	11.3	10.5	6.9	7.5	-	-	-	-	21.4	36.7	32.2	33.1
Cumulative egg number [n]					**6366**	**5325**	**4997**	**6094**												

Table 134: F_0 generation, reproduction: fecundity and fertilisation rate; 1.00 µg Dexamethasone/L (nominal), respective 0.91 µg Dexamethasone/L (mean measured)

	Nominal concentration: 1.00 µg Dexamethasone/L Mean measured concentration: 0.91 µg Dexamethasone/L																			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Time of exposure [day]	Fertilised eggs [n]				Total egg number [n]				Fertilisation rate [%]				Female number [n]				Total egg number per female [n]			
1	189	399	279	238	199	422	295	245	95.0	94.5	94.6	97.1	5	5	5	5	40	84	59	49
2	124	48	175	110	131	62	177	125	94.7	77.4	98.9	88.0	5	5	5	5	26	12	35	25
3	241	293	430	389	277	427	494	410	87.0	68.6	87.0	94.9	5	5	5	5	55	85	99	82
4	272	106	450	367	297	136	490	377	91.6	77.9	91.8	97.3	5	5	5	5	59	27	98	75
5	217	194	210	226	251	236	235	238	86.5	82.2	89.4	95.0	5	5	5	5	50	47	47	48
6	163	91	180	246	179	102	239	287	91.1	89.2	75.3	85.7	5	5	5	5	36	20	48	57
7	177	277	257	286	188	299	307	305	94.1	92.6	83.7	93.8	5	5	5	5	38	60	61	61
8	197	282	356	277	212	303	400	286	92.9	93.1	89.0	96.9	5	5	5	5	42	61	80	57
9	225	108	356	201	237	116	382	215	94.9	93.1	93.2	93.5	5	5	5	5	47	23	76	43
10	103	246	263	249	115	341	281	294	89.6	72.1	93.6	84.7	5	5	5	5	23	68	56	59
11	194	98	274	221	229	133	313	250	84.7	73.7	87.5	88.4	5	5	5	5	46	27	63	50
12	57	464	299	281	61	508	348	309	93.4	91.3	85.9	90.9	5	5	5	5	12	102	70	62
13	284	187	239	325	311	215	266	371	91.3	87.0	89.8	87.6	5	5	5	5	62	43	53	74
14	137	398	322	345	154	423	354	375	89.0	94.1	91.0	92.0	5	5	5	5	31	85	71	75
15	40	157	293	232	67	216	339	252	59.7	72.7	86.4	92.1	5	5	5	5	13	43	68	50

	Nominal concentration: 1.00 µg Dexamethasone/L **Mean measured concentration: 0.91 µg Dexamethasone/L**																			
16	144	308	251	90	213	484	292	131	67.6	63.6	86.0	68.7	5	5	5	5	43	97	58	26
17	134	200	258	350	232	313	364	434	57.8	63.9	70.9	80.6	5	5	5	5	46	63	73	87
18	194	117	301	99	210	158	345	118	92.4	74.1	87.2	83.9	5	5	5	5	42	32	69	24
19	168	93	163	209	187	107	210	232	89.8	86.9	77.6	90.1	5	5	5	5	37	21	42	46
20	135	49	325	298	162	63	340	310	83.3	77.8	95.6	96.1	5	5	5	5	32	13	68	62
Mean	**-**	**-**			**196**	**253**	**324**	**278**	**86.3**	**81.3**	**87.7**	**89.9**	**-**	**-**	**-**	**-**	**39**	**51**	**65**	**56**
SD	-	-	-	-	68	145	82	90	11.2	10.4	6.9	6.9	-	-	-	-	14	29	16	18
RSD	-	-	-	-	34.6	57.1	25.3	32.3	13.0	12.8	7.8	7.7	-	-	-	-	34.6	57.1	25.3	32.3
Cumulative egg number [n]					**3912**	**5064**	**6471**	**5564**												

Table 135: F_0 generation, reproduction: fecundity and fertilisation rate; 3.20 µg Dexamethasone/L (nominal), respective 3.19 µg Dexamethasone/L (mean measured)

	Nominal concentration: 3.20 µg Dexamethasone/L Mean measured concentration: 3.19 µg Dexamethasone/L																			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Time of exposure [day]	**Fertilised eggs [n]**				**Total egg number [n]**				**Fertilisation rate [%]**				**Female number [n]**				**Total egg number per female [n]**			
1	306	372	316	510	320	391	340	566	95.6	95.1	92.9	90.1	5	6	5	5	64	65	68	113
2	4	244	237	0	4	255	258	0	100.0	95.7	91.9	-	5	6	5	5	1	43	52	0
3	288	330	362	575	358	397	403	661	80.4	83.1	89.8	87.0	5	6	5	5	72	66	81	132
4	99	230	329	427	139	255	342	458	71.2	90.2	96.2	93.2	5	6	5	5	28	43	68	92
5	124	151	305	235	220	183	360	273	56.4	82.5	84.7	86.1	5	6	5	5	44	31	72	55
6	243	132	33	263	285	146	34	293	85.3	90.4	97.1	89.8	5	6	5	5	57	24	7	59
7	269	161	264	411	286	173	286	451	94.1	93.1	92.3	91.1	5	6	5	5	57	29	57	90
8	351	318	238	170	379	337	294	185	92.6	94.4	81.0	91.9	5	6	5	5	76	56	59	37
9	301	250	244	490	343	268	272	533	87.8	93.3	89.7	91.9	5	6	5	5	69	45	54	107
10	180	250	256	209	279	325	333	236	64.5	76.9	76.9	88.6	5	6	5	5	56	54	67	47
11	461	103	327	337	531	119	342	382	86.8	86.6	95.6	88.2	5	6	5	5	106	20	68	76
12	176	283	318	348	192	342	339	390	91.7	82.7	93.8	89.2	5	6	5	5	38	57	68	78
13	418	401	219	109	476	438	241	122	87.8	91.6	90.9	89.3	5	6	5	5	95	73	48	24
14	298	304	204	447	310	313	212	486	96.1	97.1	96.2	92.0	5	6	5	5	62	52	42	97
15	439	310	218	267	500	360	320	285	87.8	86.1	68.1	93.7	5	6	5	5	100	60	64	57

	Nominal concentration: 3.20 µg Dexamethasone/L Mean measured concentration: 3.19 µg Dexamethasone/L																			
16	436	480	184	274	527	574	235	318	82.7	83.6	78.3	86.2	5	6	5	5	105	96	47	64
17	323	131	217	349	371	156	320	387	87.1	84.0	67.8	90.2	5	6	5	5	74	26	64	77
18	311	502	317	260	364	587	389	314	85.4	85.5	81.5	82.8	5	6	5	5	73	98	78	63
19	264	199	279	192	309	211	290	227	85.4	94.3	96.2	84.6	5	6	5	5	62	35	58	45
20	334	408	350	250	375	435	370	300	89.1	93.8	94.6	83.3	5	6	5	5	75	73	74	60
Mean	**-**	**-**			**328**	**313**	**299**	**343**	**85.4**	**89.0**	**87.8**	**88.9**	**-**	**-**	**-**	**-**	**66**	**52**	**60**	**69**
SD	-	-	-	-	130	133	81	157	10.6	5.7	9.2	3.2	-	-	-	-	26	22	16	31
RSD	-	-	-	-	39.5	42.5	27.1	45.7	12.4	6.4	10.5	3.6	-	-	-	-	39.5	42.5	27.1	45.7
Cumulative egg number [n]					**6568**	**6265**	**5980**	**6867**												

Table 136: F_0 generation, reproduction: fecundity and fertilisation rate; 10.0 µg Dexamethasone/L (nominal), respective 10.5 µg Dexamethasone/L (mean measured)

	Nominal concentration: 10.0 µg Dexamethasone/L Mean measured concentration: 10.5 µg Dexamethasone/L																			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Time of exposure [day]	**Fertilised eggs [n]**				**Total egg number [n]**				**Fertilisation rate [%]**				**Female number [n]**				**Total egg number per female [n]**			
1	390	270	428	276	405	275	522	285	96.3	98.2	82.0	96.8	5	5	5	5	81	55	104	57
2	97	188	71	105	114	206	114	123	85.1	91.3	62.3	85.4	5	5	5	5	23	41	23	25
3	833	325	463	446	954	389	594	478	87.3	83.5	77.9	93.3	5	5	5	5	191	78	119	96
4	239	279	327	324	275	332	465	366	86.9	84.0	70.3	88.5	5	5	5	5	55	66	93	73
5	432	320	450	350	475	380	581	390	90.9	84.2	77.5	89.7	5	5	5	5	95	76	116	78
6	136	267	338	399	149	290	454	434	91.3	92.1	74.4	91.9	5	5	5	5	30	58	91	87
7	509	344	303	110	570	359	454	135	89.3	95.8	66.7	81.5	5	5	5	5	114	72	91	27
8	74	265	238	321	82	287	310	380	90.2	92.3	76.8	84.5	5	5	5	5	16	57	62	76
9	720	234	317	185	820	246	393	230	87.8	95.1	80.7	80.4	5	5	5	5	164	49	79	46
10	124	222	232	150	199	307	309	239	62.3	72.3	75.1	62.8	5	5	5	5	40	61	62	48
11	423	273	358	297	485	339	449	352	87.2	80.5	79.7	84.4	5	5	5	5	97	68	90	70
12	299	281	321	450	354	329	392	484	84.5	85.4	81.9	93.0	5	5	5	5	71	66	78	97
13	247	373	260	117	284	421	336	177	87.0	88.6	77.4	66.1	5	5	5	5	57	84	67	35
14	595	325	471	419	650	338	571	440	91.5	96.2	82.5	95.2	5	5	5	5	130	68	114	88
15	453	169	153	309	496	200	266	377	91.3	84.5	57.5	82.0	5	5	5	5	99	40	53	75

	Nominal concentration: 10.0 µg Dexamethasone/L **Mean measured concentration: 10.5 µg Dexamethasone/L**																			
16	309	399	333	306	399	492	505	417	77.4	81.1	65.9	73.4	5	5	5	5	80	98	101	83
17	314	306	148	397	562	413	186	449	55.9	74.1	79.6	88.4	5	5	5	5	112	83	37	90
18	208	260	120	55	278	284	188	104	74.8	91.5	63.8	52.9	5	5	5	5	56	57	38	21
19	273	315	181	505	355	327	237	561	76.9	96.3	76.4	90.0	5	5	5	5	71	65	47	112
20	255	308	156	300	325	333	206	348	78.5	92.5	75.7	86.2	5	5	5	5	65	67	41	70
Mean	**-**	**-**			**412**	**327**	**377**	**338**	**83.6**	**88.0**	**74.2**	**83.3**	**-**	**-**	**-**	**-**	**82**	**65**	**75**	**68**
SD	-	-	-	-	225	71	146	131	10.1	7.4	7.3	11.5	-	-	-	-	45	14	29	26
RSD	-	-	-	-	54.6	21.8	38.6	38.8	12.1	8.4	9.8	13.8	-	-	-	-	54.6	21.8	38.6	38.8
Cumulative egg number [n]					**8231**	**6547**	**7532**	**6769**												

Table 137: F_0 generation, reproduction: fecundity and fertilisation rate; 32.0 µg Dexamethasone/L (nominal), respective 34.7 µg Dexamethasone/L (mean measured)

	Nominal concentration: 32.0 µg Dexamethasone/L Mean measured concentration: 34.7 µg Dexamethasone/L																			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Time of exposure [day]	Fertilised eggs [n]				Total egg number [n]				Fertilisation rate [%]				Female number [n]				Total egg number per female [n]			
1	192	499	366	426	203	536	379	462	94.6	93.1	96.6	92.2	4	5	5	5	51	107	76	92
2	197	503	69	0	201	531	74	0	98.0	94.7	93.2	-	4	5	5	5	50	106	15	0
3	336	418	408	365	479	477	453	400	70.1	87.6	90.1	91.3	4	5	5	5	120	95	91	80
4	371	344	349	237	431	401	380	263	86.1	85.8	91.8	90.1	4	5	5	5	108	80	76	53
5	258	209	227	237	290	238	247	283	89.0	87.8	91.9	83.7	4	5	5	5	73	48	49	57
6	302	230	229	220	336	259	255	267	89.9	88.8	89.8	82.4	4	5	5	5	84	52	51	53
7	309	382	303	280	355	416	350	309	87.0	91.8	86.6	90.6	4	5	5	5	89	83	70	62
8	337	136	349	169	374	146	376	197	90.1	93.2	92.8	85.8	4	5	5	5	94	29	75	39
9	280	292	355	407	304	310	384	426	92.1	94.2	92.4	95.5	4	5	5	5	76	62	77	85
10	267	73	283	219	324	156	310	239	82.4	46.8	91.3	91.6	4	5	5	5	81	31	62	48
11	198	414	304	290	271	481	348	311	73.1	86.1	87.4	93.2	4	5	5	5	68	96	70	62
12	253	131	135	91	269	137	165	94	94.1	95.6	81.8	96.8	4	5	5	5	67	27	33	19
13	306	225	400	262	352	261	449	291	86.9	86.2	89.1	90.0	4	5	5	5	88	52	90	58
14	506	360	330	149	546	371	364	168	92.7	97.0	90.7	88.7	4	5	5	5	137	74	73	34
15	286	509	160	236	323	559	181	317	88.5	91.1	88.4	74.4	4	5	5	5	81	112	36	63

	Nominal concentration: 32.0 µg Dexamethasone/L **Mean measured concentration: 34.7 µg Dexamethasone/L**																			
16	205	108	426	59	307	164	558	102	66.8	65.9	76.3	57.8	4	5	5	5	77	33	112	20
17	137	372	241	315	301	623	318	381	45.5	59.7	75.8	82.7	4	5	5	5	75	125	64	76
18	156	177	271	264	215	209	352	300	72.6	84.7	77.0	88.0	4	5	5	5	54	42	70	60
19	270	178	346	189	362	220	420	228	74.6	80.9	82.4	82.9	4	5	5	5	91	44	84	46
20	270	222	298	255	360	257	358	291	75.0	86.4	83.2	87.6	4	5	5	5	90	51	72	58
Mean	**-**	**-**	**-**	**-**	**330**	**338**	**336**	**266**	**82.5**	**84.9**	**87.4**	**86.6**	**-**	**-**	**-**	**-**	**83**	**68**	**67**	**53**
SD	-	-	-	-	86	155	110	114	12.6	12.9	6.0	8.8	-	-	-	-	22	31	22	23
RSD	-	-	-	-	26.1	46.0	32.7	42.9	15.3	15.2	6.9	10.1	-	-	-	-	26.1	46.0	32.7	42.9
Cumulative egg number [n]					**6603**	**6752**	**6721**	**5329**												

Table 138: F_0 generation, reproduction: fecundity and fertilisation rate; 100 µg Dexamethasone/L (nominal), respective 100 µg Dexamethasone/L (mean measured)

	Nominal concentration: 100 µg Dexamethasone/L Mean measured concentration: 100 µg Dexamethasone/L																			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Time of exposure [day]	Fertilised eggs [n]				Total egg number [n]				Fertilisation rate [%]				Female number [n]				Total egg number per female [n]			
1	213	320	164	354	229	328	168	366	93.0	97.6	97.6	96.7	5	5	5	5	46	66	34	73
2	290	205	107	249	302	210	108	278	96.0	97.6	99.1	89.6	5	5	5	5	60	42	22	56
3	534	578	416	403	621	636	484	462	86.0	90.9	86.0	87.2	5	5	5	5	124	127	97	92
4	352	420	433	174	449	473	442	187	78.4	88.8	98.0	93.0	5	5	5	5	90	95	88	37
5	387	334	93	280	441	353	94	306	87.8	94.6	98.9	91.5	5	5	5	5	88	71	19	61
6	261	394	365	209	298	470	411	258	87.6	83.8	88.8	81.0	5	5	5	5	60	94	82	52
7	334	267	411	320	358	295	431	359	93.3	90.5	95.4	89.1	5	5	5	5	72	59	86	72
8	356	325	81	179	385	355	97	205	92.5	91.5	83.5	87.3	5	5	5	5	77	71	19	41
9	502	213	449	174	533	249	500	180	94.2	85.5	89.8	96.7	5	5	5	5	107	50	100	36
10	308	294	148	264	355	340	196	330	86.8	86.5	75.5	80.0	5	5	5	5	71	68	39	66
11	506	359	447	314	548	390	473	335	92.3	92.1	94.5	93.7	5	5	5	5	110	78	95	67
12	288	271	350	0	312	290	410	0	92.3	93.4	85.4	-	5	5	5	5	62	58	82	0
13	440	157	318	488	457	164	328	517	96.3	95.7	97.0	94.4	5	5	5	5	91	33	66	103
14	481	437	152	77	511	456	160	80	94.1	95.8	95.0	96.3	5	5	5	5	102	91	32	16
15	346	471	456	372	371	504	491	410	93.3	93.5	92.9	90.7	5	5	5	5	74	101	98	82

	Nominal concentration: 100 µg Dexamethasone/L **Mean measured concentration: 100 µg Dexamethasone/L**																			
16	418	261	320	219	457	288	359	227	91.5	90.6	89.1	96.5	5	5	5	5	91	58	72	45
17	349	114	221	483	421	175	288	509	82.9	65.1	76.7	94.9	5	5	5	5	84	35	58	102
18	280	245	284	130	319	260	337	152	87.8	94.2	84.3	85.5	5	5	5	5	64	52	67	30
19	352	138	91	243	386	156	119	260	91.2	88.5	76.5	93.5	5	5	5	5	77	31	24	52
20	300	223	252	333	330	248	282	355	90.9	89.9	89.4	93.8	5	5	5	5	66	50	56	71
Mean	**-**	**-**			**404**	**332**	**309**	**289**	**90.4**	**90.3**	**89.7**	**91.1**	**-**	**-**	**-**	**-**	**81**	**66**	**62**	**58**
SD	-	-	-	-	99	126	146	135	4.5	7.0	7.6	5.0	-	-	-	-	20	25	29	27
RSD	-	-	-	-	24.4	38.1	47.4	46.7	5.0	7.8	8.5	5.5	-	-	-	-	24.4	38.1	47.4	46.7
Cumulative egg number [n]					**8083**	**6640**	**6178**	**5776**												

Table 139: F_0 generation, summary: reproduction

		Nominal concentration Dexamethasone [µg/L]						
		Control	**0.32**	**1.00**	**3.20**	**10.0**	**32.0**	**100**
		Mean measured concentration Dexamethasone [µg/L]						
	Replicate	**Control**	**0.33**	**0.91**	**3.19**	**10.5**	**34.7**	**100**
Total eggs per day and female [n]	A	83	64	39	66	82	83	81
	B	70	53	51	52	65	68	66
	C	65	50	65	60	75	67	62
	D	81	61	56	69	68	53	58
	Mean	**75**	**57(*)**	**53(*)**	**62**	**73**	**68**	**67**
	SD	9	6	11	7	8	12	10
	RSD	11.6	11.3	20.3	11.8	10.6	17.7	15.1
Fertilisation rate [%]	A	90.8	83.6	86.3	85.4	83.6	82.5	90.4
	B	94.2	88.4	81.3	89.0	88.0	84.9	90.3
	C	88.0	90.3	87.7	87.8	74.2	87.4	89.7
	D	91.3	86.5	89.9	88.9	83.3	86.6	91.1
	Mean	**91.1**	**87.2**	**86.3**	**87.8**	**82.3(*)**	**85.3(*)**	**90.4**
	SD	2.5	2.9	3.6	1.7	5.8	2.2	0.6
	RSD	2.8	3.3	4.2	1.9	7.0	2.6	0.7

(*) Statistically significant reduction compared to control, p<0.05, Dunnett`s test, one-sided smaller.

C.2.5 Parental generation (F_0): total length and wet weight at termination

Table 140: F_0 generation, individual total length at termination [cm]; control and 0.32 µg Dexamethasone/L (nominal), respective 0.33 µg Dexamethasone/L (mean measured)

	Control								Nominal concentration: 0.32 µg Dexamethasone/L Mean measured concentration: 0.33 µg Dexamethasone/L							
	Total length [cm]															
Replicate	A		B		C		D		A		B		C		D	
Fish No.	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	-	3.7	-	4.4	3.8	-	3.8	-	-	4.7	3.9	-	4.0	-	3.7	-
2	4.1	-	3.8	-	3.5	-	-	4.0	-	3.7	-	4.3	-	4.0	3.5	-
3	-	4.0	-	4.0	-	3.7	3.9	-	-	3.6	4.0	-	4.0	-	-	3.6
4	-	4.1	3.7	-	3.6	-	-	3.9	-	3.9	-	4.2	-	4.0	3.8	-
5	3.9	-	-	3.9	3.9	-	3.8	-	-	3.9	-	4.5	4.0	-	-	3.7
6	-	4.1	3.6	-	-	4.0	3.9	-	4.0	-	3.8		-	4.2	3.5	-
7	3.7	-	-	4.0	3.6	-	-	3.8	4.0	-	-	4.0	3.9	-	-	3.6
8	3.6	-	3.5	-	-	4.0	3.8	-	3.8	-	3.6	-	-	3.7	3.5	-
9	-	4.0	-	3.6	-	4.2	-	4.0	4.0	-	-	3.8	3.7	-	-	3.6
10	3.9	-	3.6	-	-	3.8	-	3.6	3.5	-	4.0	-	-	3.6	-	3.5
Mean	**3.8**	**4.0**	**3.6**	**4.0**	**3.7**	**3.9**	**3.8**	**3.9**	**3.9**	**4.0**	**3.9**	**4.2**	**3.9**	**3.9**	**3.6**	**3.6**
SD	0.2	0.2	0.1	0.3	0.2	0.2	0.1	0.2	0.2	0.4	0.2	0.3	0.1	0.2	0.1	0.1
RSD	5.1	4.1	3.1	7.2	4.5	4.9	1.4	4.3	5.7	10.9	4.3	6.5	3.3	6.3	3.9	2.0

Table 141: F_0 generation, individual total length at termination [cm]; 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.91 and 3.19 µg Dexamethasone/L (mean measured)

	Nominal concentration: 1.00 µg Dexamethasone/L Mean measured concentration: 0.91 µg Dexamethasone/L								Nominal concentration: 3.20 µg Dexamethasone/L Mean measured concentration: 3.19 µg Dexamethasone/L							
	Total length [cm]															
Replicate	**A**		**B**		**C**		**D**		**A**		**B**		**C**		**D**	
Fish No.	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
1	3.7	-	3.8	-	3.7	-	-	3.9	3.8	-	4.0	-	4.0	-	3.8	-
2	-	3.5	-	4.4	-	3.9	-	4.2	3.5	-	-	4.0	-	4.3	-	3.9
3	-	4.3	3.7	-	3.6	-	-	3.8	-	3.9	-	4.2	-	4.0	-	3.9
4	3.8	-	-	4.2	-	3.6	-	4.0	4.0	-	3.8	-	4.0	-	3.8	-
5	-	4.0	-	3.7	3.6	-	-	3.4	3.5	-	-	4.0		4.1	4.0	-
6	3.7	-	3.8	-	-	3.6	3.6	-	-	3.6	3.6	-	3.9	-	-	3.7
7	-	3.7	-	4.0	3.7	-	3.8	-	3.9	-	-	3.6	3.6	-	-	3.5
8	-	4.0	3.8	-	4.0	-	3.5	-	-	3.8	4.0	-	3.9	-	-	3.6
9	3.8	-	-	3.3	-	3.7	3.7	-	-	3.5	-	3.8	-	4.0	3.8	-
10	3.8	-	4.0	-	-	3.7	3.8	-	-	4.2	-	3.6	-	3.5	3.5	-
Mean	**3.8**	**3.9**	**3.8**	**3.9**	**3.7**	**3.7**	**3.7**	**3.9**	**3.7**	**3.8**	**3.9**	**3.9**	**3.9**	**4.0**	**3.8**	**3.7**
SD	0.1	0.3	0.1	0.4	0.2	0.1	0.1	0.3	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.2
RSD	1.5	7.9	2.9	11.0	4.4	3.3	3.5	7.7	6.2	7.2	5.0	6.3	4.2	7.4	4.7	4.8

Table 142: F_0 generation, individual total length at termination [cm]; 10.0 and 32.0 µg Dexamethasone/L (nominal), respective 10.5 and 34.7 µg Dexamethasone (mean measured)

	Nominal concentration: 10.0 µg Dexamethasone/L Mean measured concentration: 10.5 µg Dexamethasone/L								Nominal concentration: 32.0 µg Dexamethasone/L Mean measured concentration: 34.7 µg Dexamethasone/L							
	Total length [cm]															
Replicate	A		B		C		D		A		B		C		D	
Fish No.	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	3.7	-	-	4.1	3.5	-	-	4.2	3.7	-	3.6			4.0	3.7	
2	-	4.0	3.9	-	-	4.0	3.7	-	3.9	-	3.7		3.7			3.5
3	3.8	-	-	3.8	-	3.6	-	4.0	3.7	-	3.7			4.0		3.7
4	3.5	-	3.9	-	3.5	-	3.6	-	3.4	-		3.9	3.6		3.8	
5	3.5	-	-	3.8	-	4.2	-	4.0	-	3.8		3.8		3.7	3.5	
6	-	3.9	-	3.7	3.6	-	4.0	-	-	4.0	3.7		3.5			5.0
7	-	4.0	4.0	-	3.5	-	-	4.0	3.5	-		3.7		3.2	3.5	
8	-	3.8	-	3.3	3.8	-	3.5	-	-	3.9	3.7		3.7			4.0
9	-	3.7	3.7	-	-	3.9	-	4.0	-	3.8		3.7		3.5		3.7
10	3.6		3.6	-	-	3.6	3.7	-	-	-		4.0	3.5		3.8	
Mean	**3.6**	**3.9**	**3.8**	**3.7**	**3.6**	**3.9**	**3.7**	**4.0**	**3.6**	**3.9**	**3.7**	**3.8**	**3.6**	**3.7**	**3.7**	**4.0**
SD	0.1	0.1	0.2	0.3	0.1	0.3	0.2	0.1	0.2	0.1	0.0	0.1	0.1	0.3	0.2	0.6
RSD	3.6	3.4	4.3	7.7	3.6	6.8	5.1	2.2	5.4	2.5	1.2	3.4	2.8	9.3	4.1	15.0

Table 143: F_0 generation, individual total length at termination [cm]; 100 µg Dexamethasone/L (nominal), respective 100 µg Dexamethasone/L (mean measured)

	Nominal concentration: Mean measured concentration:	**100 µg Dexamethasone/L 100 µg Dexamethasone/L**						
	Total length [cm]							
Replicate	**A**		**B**		**C**		**D**	
Fish No.	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
1	3.8	-	3.5	-	-	3.9	3.7	-
2	-	3.7	3.7	-	-	3.7	-	3.8
3	-	4.0	3.7	-	-	3.8	-	3.5
4	4.0	-	3.7	-	-	3.5	-	3.9
5	-	3.5	-	3.7	-	3.8	3.5	-
6	3.7	-	-	3.9	3.5	-	-	3.6
7	-	3.8	-	4.0	3.8	-	3.5	-
8	3.6	-	-	3.9	4.0	-	-	3.5
9	-	3.7	-	3.9	3.3	-	3.4	-
10	3.6	-	-	-	3.4	-	3.7	-
Mean	**3.7**	**3.7**	**3.7**	**3.9**	**3.6**	**3.7**	**3.6**	**3.7**
SD	0.2	0.2	0.1	0.1	0.3	0.2	0.1	0.2
RSD	4.5	4.9	2.7	2.8	8.1	4.1	3.8	5.0

Table 144: F_0 generation, individual wet weight at termination [g]; control and 0.32 µg Dexamethasone/L (nominal), respective 0.33 µg Dexamethasone/L (mean measured)

	Control								Nominal concentration: 0.32 µg Dexamethasone/L Mean measured concentration: 0.33 µg Dexamethasone/L							
	Wet weight [g]															
Replicate	**A**		**B**		**C**		**D**		**A**		**B**		**C**		**D**	
Fish No.	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
1	-	0.725	-	1.030	0.532	-	0.444	-	-	1.195	0.494	-	0.477	-	0.478	-
2	0.574	-	0.452	-	0.417	-	-	0.695	-	0.585	-	0.998	-	0.732	0.358	-
3	-	0.826	-	0.793	-	0.641	0.591	-	-	0.523	0.584	-	0.571	-	-	0.682
4	-	0.710	0.457	-	0.542	-	-	0.616	-	0.717	-	0.852	-	0.629	0.514	-
5	0.486	-	-	0.803	0.534	-	0.541	-	-	0.680	-	1.045	0.518	-	-	0.529
6	-	0.722	0.441	-	-	0.696	0.489	-	0.577	-	0.469	-	-	0.842	0.442	-
7	0.455	-	-	0.671	0.508	-	-	0.583	0.521	-	-	0.611	0.535	-	-	0.530
8	0.380	-	0.386	-	-	0.749	0.495	-	0.437	-	0.397	-	-	0.724	0.435	-
9	-	0.699	-	0.510	-	0.646	-	0.778	0.478	-	-	0.554	0.462	-	-	0.502
10	0.475	-	0.435	-	-	0.623	-	0.556	0.364	-	0.524	-	-	0.538	-	0.476
Mean	**0.474**	**0.736**	**0.434**	**0.761**	**0.507**	**0.671**	**0.512**	**0.646**	**0.475**	**0.740**	**0.494**	**0.812**	**0.513**	**0.693**	**0.445**	**0.544**
SD	0.070	0.051	0.028	0.191	0.052	0.051	0.056	0.091	0.081	0.266	0.069	0.222	0.044	0.115	0.058	0.080
RSD	14.7	6.9	6.5	25.1	10.2	7.6	10.9	14.0	17.1	35.9	14.0	27.4	8.6	16.6	13.1	14.8

Table 145: F_0 generation, individual wet weight at termination [g]; 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.91 and 3.19 µg Dexamethasone/L (mean measured)

	Nominal concentration: 1.00 µg Dexamethasone/L Mean measured concentration: 0.91 µg Dexamethasone/L								Nominal concentration: 3.20 µg Dexamethasone/L Mean measured concentration: 3.19 µg Dexamethasone/L							
	Wet weight [g]															
Replicate	**A**		**B**		**C**		**D**		**A**		**B**		**C**		**D**	
Fish No.	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
1	0.516	-	0.525	-	0.475	-	-	0.740	0.462	-	0.575		0.468	-	0.486	-
2	-	0.478	-	1.170	-	0.814	-	0.907	0.399	-	-	0.771	-	0.888	-	0.765
3	-	1.089	0.455	-	0.407	-	-	0.590	-	0.826	-	0.844	-	0.729	-	0.581
4	0.531	-	-	0.660	-	0.559	-	0.792	0.555	-	0.460		0.584	-	0.529	-
5	-	0.736	-	0.563	0.435	-	-	0.482	0.427	-	-	0.726	-	0.886	0.542	-
6	0.441	-	0.466	-	-	0.523	0.418	-	-	0.641	0.431		0.500	-	-	0.596
7	-	0.571	-	0.699	0.466	-	0.508	-	0.483	-	-	0.553	0.426	-	-	0.513
8	-	0.779	0.470	-	0.507	-	0.415	-	-	0.747	0.556		0.560	-	-	0.585
9	0.447	-	-	0.463	-	0.565	0.482	-	-	0.455	-	0.764	-	0.550	0.453	-
10	0.372	-	0.511	-	-	0.606	0.453	-	-	0.800	-	0.439	-	0.516	0.315	-
Mean	**0.461**	**0.731**	**0.485**	**0.711**	**0.458**	**0.613**	**0.455**	**0.702**	**0.465**	**0.694**	**0.506**	**0.683**	**0.508**	**0.714**	**0.465**	**0.608**
SD	0.064	0.235	0.031	0.272	0.038	0.116	0.040	0.168	0.060	0.151	0.071	0.154	0.065	0.178	0.091	0.094
RSD	13.9	32.1	6.3	38.3	8.4	18.9	8.9	23.9	12.8	21.8	14.0	22.5	12.8	24.9	19.6	15.4

Table 146: F_0 generation, individual wet weight at termination [g]; 10.0 and 32.0 µg Dexamethasone/L (nominal), respective 10.5 and 34.7 µg Dexamethasone (mean measured)

	Nominal concentration: 10.0 µg Dexamethasone/L Mean measured concentration: 10.5 µg Dexamethasone/L								Nominal concentration: 32.0 µg Dexamethasone/L Mean measured concentration: 34.7 µg Dexamethasone/L							
	Wet weight [g]															
Replicate	**A**		**B**		**C**		**D**		**A**		**B**		**C**		**D**	
Fish No.	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
1	0.420	-	-	0.860	0.361	-	-	0.777	0.432	-	0.391	-	-	0.669	0.438	-
2	-	0.716	0.513	-	-	0.731	0.429	-	0.454	-	0.461	-	0.478	-	-	0.480
3	0.426	-	-	0.566	-	0.662	-	0.772	0.452	-	0.443	-	-	0.669	-	0.645
4	0.440	-	0.540	-	0.362	-	0.350	-	0.360	-	-	0.723	0.471	-	0.486	-
5	0.396	-	-	0.566	-	0.779	-	0.599	-	0.639	-	0.602	-	0.626	0.387	-
6	-	0.645	-	0.615	0.372	-	0.550	-	-	0.741	0.420	-	0.454	-	-	1.117
7	-	0.682	0.600	-	0.316	-	-	0.706	0.411	-	-	0.584	-	0.403	0.375	-
8	-	0.605	-	0.445	0.434	-	0.491	-	-	0.678	0.488	-	0.388	-	-	0.706
9	-	0.530	0.470	-	-	0.602	-	0.712	-	0.619	-	0.507	-	0.470	-	0.566
10	0.399	-	0.416	-	-	0.475	0.397	-	-	-	-	0.653	0.379	-	0.437	-
Mean	**0.416**	**0.636**	**0.508**	**0.610**	**0.369**	**0.650**	**0.443**	**0.713**	**0.422**	**0.669**	**0.441**	**0.614**	**0.434**	**0.567**	**0.425**	**0.703**
SD	0.019	0.072	0.070	0.153	0.042	0.119	0.079	0.072	0.039	0.054	0.037	0.080	0.047	0.123	0.045	0.247
RSD	4.5	11.3	13.7	25.1	11.5	18.3	17.7	10.1	9.2	8.0	8.5	13.1	10.8	21.7	10.5	35.1

Table 147: F_0 generation, individual wet weight at termination [g]; 100 µg Dexamethasone/L (nominal), respective 100 µg Dexamethasone/L (mean measured)

	Nominal concentration: 100 µg Dexamethasone/L Mean measured concentration: 100 µg Dexamethasone/L							
	Wet weight [g]							
Replicate	**A**		**B**		**C**		**D**	
Fish No.	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
1	0.428	-	0.354	-	-	0.569	0.456	-
2	-	0.559	0.413	-	-	0.510	-	0.622
3	-	0.575	0.371	-	-	0.558	-	0.502
4	0.571	-	0.364	-	-	0.501	-	0.588
5	-	0.476	-	0.563	-	0.581	0.407	-
6	0.445	-	-	0.689	0.383	-	-	0.520
7	-	0.534	-	0.612	0.485	-	0.351	-
8	0.430	-	-	0.651	0.464	-	-	0.438
9	-	0.579	-	0.555	0.333	-	0.334	-
10	0.440	-	-	-	0.286	-	0.426	-
Mean	**0.463**	**0.545**	**0.376**	**0.614**	**0.390**	**0.544**	**0.395**	**0.534**
SD	0.061	0.042	0.026	0.057	0.085	0.036	0.051	0.073
RSD	13.2	7.8	6.9	9.3	21.7	6.6	13.0	13.6

Table 148: F_0 generation, summary: mean total length and mean wet weight at termination

	Nominal concentration Dexamethasone [µg/L] (Mean measured concentration Dexamethasone [µg/L])							
	Replicate	**Control**	**0.32 (0.33)**	**1.00 (0.91)**	**3.20 (3.19)**	**10.0 (10.5)**	**32.0 (34.7)**	**100 (100)**
Total length males at termination [cm]	A	3.8	3.9	3.8	3.7	3.6	3.6	3.7
	B	3.6	3.9	3.8	3.9	3.8	3.7	3.7
	C	3.7	3.9	3.7	3.9	3.9	3.6	3.6
	D	3.8	3.6	3.7	3.8	3.7	3.7	3.6
	Mean	**3.8**	**3.8**	**3.7**	**3.8**	**3.8**	**3.6**	**3.6**
	SD	0.1	0.1	0.1	0.1	0.1	0.0	0.1
	RSD	2.8	3.7	1.6	1.7	2.9	0.9	2.1
Total length females at termination [cm]	A	4.0	4.0	3.9	3.8	3.9	3.9	3.7
	B	4.0	4.2	3.9	3.9	3.7	3.8	3.9
	C	3.9	3.9	3.7	4.0	3.9	3.7	3.7
	D	3.9	3.6	3.9	3.7	4.0	4.0	3.7
	Mean	**3.9**	**3.9**	**3.8**	**3.8**	**3.9**	**3.8**	**3.8(**)**
	SD	0.1	0.2	0.1	0.1	0.1	0.1	0.1
	RSD	1.4	5.9	2.6	2.9	3.2	3.3	2.4
Wet weight males at termination [g]	A	0.474	0.475	0.461	0.465	0.416	0.422	0.463
	B	0.434	0.494	0.485	0.506	0.508	0.441	0.376
	C	0.507	0.513	0.458	0.508	0.650	0.434	0.390
	D	0.512	0.445	0.455	0.465	0.443	0.425	0.395
	Mean	**0.482**	**0.482**	**0.465**	**0.486**	**0.504**	**0.430(*)**	**0.406(*)**
	SD	0.036	0.029	0.014	0.024	0.104	0.009	0.039
	RSD	7.4	5.9	3.0	4.9	20.7	2.0	9.6
Wet weight females at termination [g]	A	0.736	0.740	0.731	0.694	0.636	0.669	0.545
	B	0.761	0.812	0.711	0.683	0.610	0.614	0.614
	C	0.671	0.693	0.613	0.714	0.650	0.567	0.544
	D	0.646	0.544	0.702	0.608	0.713	0.703	0.534
	Mean	**0.704**	**0.697**	**0.689**	**0.675**	**0.652**	**0.638**	**0.559(**)**
	SD	0.054	0.113	0.052	0.046	0.044	0.060	0.037
	RSD	7.7	16.3	7.5	6.9	6.7	9.4	6.6

(*) Statistically significant reduction compared to conrol, p<0.05, Jonckheere-Terpstra test, one-sided smaller.

(**) Statistically significant reduction compared to conrol, p<0.05, Williams test, one-sided smaller.

Table 149: F_0 generation, sex ratio, control, 0.32, 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.33, 0.91 and 3.19 µg Dexamethasone/L (mean measured) (based on histological data provided in C.2.13)

	Nominal concentration Dexamethasone [µg/L]															
	Control				0.32				1.00				3.20			
	Mean measured concentration Dexamethasone [µg/L]															
	Control				0.33				0.91				3.19			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Male [n]	5	5	5	5	5	5	5	5	5	5	5	5	5	4	5	5
Female [n]	5	5	5	5	5	5	5	5	5	5	5	5	5	6	5	5
Total [n]	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Male [%]	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	40.0	50.0	50.0
Female [%]	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	60.0	50.0	50.0
Total [%]	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Total mean male [%]				50.0				50.0				50.0				47.5
SD				0.0				0.0				0.0				5.0
RSD				0.0				0.0				0.0				10.5
Total mean female [%]				50.0				50.0				50.0				52.5
SD				0.0				0.0				0.0				5.0
RSD				0.0				0.0				0.0				9.5

Table 150: F_0 generation, sex ratio, 10.0, 32.0 and 100 µg Dexamethasone/L (nominal), respective 10.5, 34.7 and 100 µg Dexamethasone/L (mean measured) (based on histological data provided in C.2.14)

	Nominal concentration Dexamethasone [µg/L]											
	10.0				**32.0**				**100**			
	Mean measured concentration Dexamethasone [µg/L]											
	10.5				**34.7**				**100**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Male [n]	5	5	5	5	5	5	5	5	5	4	5	5
Female [n]	5	5	5	5	4	5	5	5	5	5	5	5
Total [n]	10	10	10	10	9	10	10	10	10	9	10	10
Male [%]	50.0	50.0	50.0	50.0	55.6	50.0	50.0	50.0	50.0	44.4	50.0	50.0
Female [%]	50.0	50.0	50.0	50.0	44.4	50.0	50.0	50.0	50.0	55.6	50.0	50.0
Total [%]	100	100	100	100	100	100	100	100	100	100	100	100
Total mean male [%]	**50.0**				**51.4**				**48.6**			
SD	0.0				2.8				2.8			
RSD	0.0				5.4				5.7			
Total mean female [%]	**50.0**				**48.6**				**51.4**			
SD	0.0				2.8				2.8			
RSD	0.0				5.7				5.4			

C.2.6 First filial generation (F_1), early life stage

Table 151: F_1 generation, early life stage: hatching success, survival at day 21 and 35 dpf

		Nominal concentration Dexamethasone [µg/L]						
		Control	**0.32**	**1.00**	**3.20**	**10.0**	**32.0**	**100**
		Mean measured concentration Dexamethasone [µg/L]						
	Replicate	**Control**	**0.33**	**0.91**	**3.19**	**10.5**	**34.7**	**100**
Introduced eggs [n]	A	36	36	36	36	36	36	36
	B	36	36	36	36	36	36	36
	C	36	36	36	36	36	36	36
	D	36	36	36	36	36	36	36
Hatch, day 2 pf [n]	A	5	3	3	3	7	8	8
	B	5	3	2	1	3	7	9
	C	4	4	4	3	8	3	6
	D	11	3	4	3	10	6	10
Hatch, day 3 pf [n]	A	21	34	16	27	19	30	34
	B	15	19	17	25	17	33	33
	C	20	16	21	24	21	30	29
	D	26	24	16	23	23	31	34
Hatch, day 4 pf [n]	A	34	34	33	34	35	30	36
	B	33	34	33	34	35	33	36
	C	36	34	36	36	27	31	36
	D	35	27	31	35	30	34	36
Hatch, day 5 pf [n]	A	36	34	35	36	36	31	36
	B	34	36	34	35	36	34	36
	C	36	35	36	36	27	32	36
	D	36	29	32	36	30	36	36
Hatch, day 5 pf [%]	A	100	94.4	97.2	100	100	86.1	100
	B	94.4	100	94.4	97.2	100.0	94.4	100
	C	100	97.2	100	100	75.0	88.9	100
	D	100	80.6	88.9	100	83.3	100	100
	Mean	**98.6**	**93.1**	**95.1**	**99.3**	**89.6**	**92.4**	**100**
	SD	2.8	8.6	4.7	1.4	12.5	6.2	0.0
	RSD	2.8	9.3	5.0	1.4	14.0	6.7	0.0

		Nominal concentration Dexamethasone [µg/L]						
Survived, day 21 pf [n]	A	36	34	35	36	35	31	36
	B	-(*)	36	34	35	36	34	34
	C	36	35	36	36	27	32	36
	D	33	29	32	35	30	36	36
Survived, day 21 pf [%]	A	100	100	100	100	97.2	100	100
	B	-(*)	100	100	100	100	100	94.4
	C	100	100	100	100	100	100	100
	D	91.7	100.0	100	97.2	100	100	100
	Mean	**97.2**	**100**	**100**	**99.3**	**99.3**	**100**	**98.6**
	SD	4.8	0.0	0.0	1.4	1.4	0.0	2.8
	RSD	4.9	0.0	0.0	1.4	1.4	0.0	2.8
Survived, day 35 pf [n]	A	36	30	35	36	34	31	36
	B	-(*)	36	33	34	36	34	34
	C	36	35	36	36	20	32	36
	D	30	27	31	33	28	36	36
Survived, day 35 pf [%]	A	100	88.2	100	100	94.4	100	100
	B	-(*)	100	97.1	97.1	100	100	94.4
	C	100.0	100	100	100	74.1	100	100
	D	83.3	93.1	96.9	91.7	93.3	100	100
	Mean	**94.4**	**95.3**	**98.5**	**97.2**	**90.5**	**100**	**98.6**
	SD	9.6	5.7	1.8	3.9	11.3	0.0	2.8
	RSD	10.2	6.0	1.8	4.0	12.5	0.0	2.8

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

Table 152: F_1 generation, early life stage: individual total length at day 35 pf [cm]; control, 0.32 and 1.00 µg Dexamethasone/L (nominal), respective 0.33 and 0.91 µg Dexamethasone/L (mean measured)

	Nominal concentration Dexamethasone [µg/L]											
	Control				**0.32**				**1.00**			
	Mean measured concentration Dexamethasone [µg/L]											
	Control				**0.33**				**0.91**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Individual total length day 35 pf [cm]**											
1	1.87	-(*)	1.98	2.07	1.75	1.59	1.69	1.81	1.73	1.87	1.83	2.00
2	1.63	-(*)	2.09	1.88	1.76	1.85	1.72	1.80	1.85	2.05	1.60	1.57
3	1.82	-(*)	1.79	2.13	2.11	1.63	1.93	1.63	2.14	1.61	1.64	1.91
4	1.99	-(*)	1.53	1.31	1.95	1.90	1.92	1.46	1.71	1.57	1.53	1.91
5	1.92	-(*)	1.69	1.84	1.98	1.82	1.76	1.82	2.12	1.68	1.94	2.01
6	1.93	-(*)	1.60	1.61	1.92	1.64	1.72	1.96	2.21	2.18	1.76	1.88
7	1.66	-(*)	2.10	1.65	1.38	2.17	1.97	2.04	2.04	1.48	1.47	1.81
8	1.35	-(*)	2.03	1.41	1.59	1.78	1.63	2.06	1.63	2.01	1.76	1.92
9	1.92	-(*)	2.19	1.64	2.04	2.00	2.00	1.90	1.63	1.62	1.71	1.66
10	1.87	-(*)	1.66	1.89	2.10	1.94	1.88	2.12	1.75	1.95	1.73	1.95
11	1.65	-(*)	1.72	1.85	2.11	1.65	1.65	2.03	2.06	1.95	1.96	1.78
12	1.93	-(*)	1.90	1.81	1.50	1.33	1.48	2.19	1.85	1.79	1.59	1.62
13	2.05	-(*)	1.79	1.50	1.72	1.84	1.86	1.98	1.78	1.96	2.14	2.09
14	1.87	-(*)	1.64	1.68	1.57	2.01	1.79	2.01	1.81	1.92	1.76	1.90
15	1.54	-(*)	1.75	2.04	1.83	1.93	1.83	1.65	1.98	1.80	1.89	2.03

	Nominal concentration Dexamethasone [µg/L]											
16	1.71	-(*)	1.86	1.96	1.38	1.58	1.99	1.85	2.01	1.80	2.02	1.88
17	1.65	-(*)	1.94	1.74	2.15	1.79	1.92	2.21	2.04	1.79	1.99	2.10
18	1.87	-(*)	1.46	1.73	2.05	1.53	2.07	1.60	2.18	1.69	2.02	1.98
19	1.73	-(*)	1.62	1.70	1.56	1.70	1.84	1.96	1.75	2.21	2.05	1.91
20	2.08	-(*)	2.01	2.13	2.21	1.99	1.49	2.04	1.92	1.80	2.03	2.15
21	1.20	-(*)	2.10	1.10	1.10	1.73	1.40	1.95	1.65	2.08	1.77	1.31
22	2.07	-(*)	1.70	2.05	1.89	1.10	0.97	1.90	1.58	1.46	1.56	1.85
23	1.52	-(*)	1.42	2.14	1.79	1.76	1.28	2.07	1.95	1.39	1.68	1.22
24	1.48	-(*)	1.59	0.96	1.78	1.96	1.24	2.04	1.62	1.57	1.78	1.69
25	1.30	-(*)	1.80	2.14	2.15	1.07	1.76	1.60	1.48	1.49	1.76	1.46
26	1.75	-(*)	1.06	1.63	1.19	1.85	2.01	1.40	1.72	1.67	1.30	1.40
27	1.54	-(*)	1.99	1.81	1.86	1.93	1.56	2.07	1.95	1.60	1.68	1.59
28	2.01	-(*)	1.43	1.52	1.49	1.65	2.11	-	1.86	1.30	1.48	1.51
29	1.29	-(*)	1.68	2.12	1.60	1.76	1.73	-	1.90	1.50	1.42	1.79
30	1.88	-(*)	1.41	1.86	1.97	1.68	2.30	-	1.69	1.63	1.31	1.73
31	1.97	-(*)	1.61	-	-	1.97	1.67	-	2.12	2.10	1.43	1.39
32	1.90	-(*)	1.82	-	-	2.02	1.68	-	1.34	1.53	1.93	-
33	2.02	-(*)	2.17	-	-	1.73	1.86	-	1.71	1.69	1.33	-
34	1.95	-(*)	1.77	-	-	1.82	1.47	-	1.58	-	1.59	-
35	1.61	-(*)	1.97	-	-	2.26	2.12	-	1.27	-	1.72	-
36	1.66	-(*)	1.95	-	-	1.65	-	-	-	-	1.77	-
37	1.85	-(*)	-	-	-	-	-	-	-	-	1.43	-

	Nominal concentration Dexamethasone [µg/L]											
38	1.60	-(*)	-	-	-	-	-	-	-	-	-	-
39	1.95	-(*)	-	-	-	-	-	-	-	-	-	-
Mean	**1.76**	-(*)	**1.77**	**1.76**	**1.78**	**1.77**	**1.75**	**1.89**	**1.82**	**1.75**	**1.71**	**1.77**
SD	0.23	-	0.25	0.30	0.30	0.25	0.27	0.21	0.23	0.24	0.23	0.25
RSD	13.2	-	14.1	17.2	16.5	14.1	15.7	11.3	12.7	13.5	13.2	13.9
Total mean				**1.76**				**1.80**				**1.76**
Total SD				0.01				0.06				0.04
Total RSD				0.4				3.6				2.5

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

Table 153: F_1 generation, early life stage: individual total length at day 35 pf [cm]; 3.20, 10.0, 32.0 and 100 µg Dexamethasone/L (nominal), respective 3.19, 10.5, 34.7 and 100 µg Dexamethasone/L (mean measured)

	Nominal concentration Dexamethasone [µg/L]															
	3.20				10.0				32.0				100			
	Mean measured concentration Dexamethasone [µg/L]															
	3.19				10.5				34.7				100			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Individual total length day 35 pf [cm]															
1	1.93	2.09	1.56	1.86	1.65	1.62	1.76	1.88	1.64	1.83	1.58	1.97	1.91	1.71	1.60	1.91
2	1.88	2.24	1.69	2.07	1.75	1.57	2.09	1.89	1.83	1.74	1.44	1.74	1.64	1.77	1.73	1.67
3	1.65	1.90	1.67	1.92	1.65	1.75	2.27	2.09	1.70	1.67	1.74	1.82	1.85	1.75	1.52	1.77
4	1.75	1.85	1.91	1.85	1.92	1.67	1.93	1.70	1.70	1.98	1.49	2.06	1.42	1.72	1.51	1.73
5	1.24	1.99	1.60	1.87	1.85	1.92	1.92	1.92	1.99	1.80	2.14	2.08	1.83	1.70	1.52	1.72
6	1.41	1.70	1.91	2.26	1.92	1.82	2.16	2.05	1.80	1.72	1.97	2.01	1.93	1.57	1.62	1.63
7	2.22	2.03	1.98	1.85	1.89	1.97	2.15	1.89	1.96	1.65	1.92	1.79	1.62	1.60	1.44	1.72
8	1.96	1.66	1.83	2.17	1.51	1.86	2.14	2.07	1.94	1.76	1.69	1.54	1.51	1.37	1.42	1.59
9	1.67	2.16	1.57	1.70	1.64	1.79	2.09	1.78	1.87	1.85	1.61	1.64	1.43	1.42	1.54	1.46
10	1.56	2.23	1.95	2.34	1.81	1.78	2.12	1.86	2.02	1.60	1.90	1.82	1.75	1.58	1.50	1.58
11	1.81	2.22	1.61	1.65	1.94	1.77	2.04	1.57	1.88	1.89	1.82	1.51	1.77	1.93	1.90	1.83
12	1.83	1.87	1.81	1.54	2.04	1.74	2.23	1.64	1.82	1.68	2.01	1.80	1.84	1.86	1.52	1.86
13	1.83	1.71	1.51	1.87	1.99	1.84	1.82	1.66	1.88	1.74	1.46	1.85	1.51	1.77	1.49	1.60
14	1.71	1.78	1.59	1.72	1.76	1.60	1.68	2.04	1.75	2.11	1.71	1.63	1.36	1.85	1.73	1.61
15	1.75	1.70	1.84	2.00	1.90	1.72	2.05	1.64	2.05	1.77	1.86	1.62	1.65	2.04	1.88	1.52
16	1.56	2.10	1.37	1.70	2.07	1.52	1.82	1.89	1.92	1.91	1.76	1.79	1.77	1.69	1.41	1.67

	Nominal concentration Dexamethasone [µg/L]															
17	1.80	1.67	1.97	2.15	1.75	1.80	2.11	1.97	1.77	1.74	1.68	1.70	1.80	1.85	1.80	1.66
18	2.01	1.84	2.05	1.82	1.50	1.99	1.73	1.80	2.05	1.67	1.57	1.74	1.50	1.66	1.59	1.63
19	1.71	1.99	1.65	1.91	1.50	1.70	1.90	1.64	1.74	1.69	1.89	1.61	1.49	1.66	1.85	1.63
20	1.83	2.12	2.20	1.65	1.94	1.65	2.17	1.90	1.62	1.84	2.03	1.89	1.66	1.93	1.52	1.58
21	1.24	1.87	1.73	1.49	1.66	1.84	-	1.26	1.77	1.95	1.35	1.76	1.44	1.60	1.70	1.52
22	1.53	1.38	1.73	1.33	1.59	1.61	-	1.71	1.73	1.58	1.41	1.59	1.63	1.58	1.55	1.59
23	2.05	1.48	1.77	1.54	1.64	2.06	-	1.49	1.56	1.68	1.40	1.65	1.47	1.55	1.40	1.44
24	1.55	1.69	1.69	1.63	1.82	1.88	-	2.05	1.53	1.66	1.33	1.51	1.40	1.31	1.71	1.71
25	1.80	1.78	1.70	1.31	1.78	1.72	-	1.81	1.95	1.62	1.98	1.38	1.38	1.63	1.65	1.59
26	2.02	1.11	1.71	1.63	1.79	1.60	-	1.67	1.54	1.68	1.59	1.78	1.34	1.82	1.51	1.54
27	1.61	1.17	1.84	1.70	1.65	1.86	-	1.20	1.86	1.72	1.22	1.48	1.51	1.78	1.41	1.37
28	1.83	1.70	1.66	1.73	2.06	1.63	-	1.61	1.90	1.39	1.87	1.61	1.59	1.74	1.50	1.55
29	1.46	1.98	1.53	1.25	1.51	1.58	-	-	1.95	1.87	1.80	1.44	1.79	1.78	1.81	1.65
30	0.92	1.64	1.56	1.66	1.69	2.06	-	-	1.75	1.45	1.47	1.79	1.81	1.62	1.29	1.36
31	1.87	1.50	1.18	1.64	1.63	1.67	-	-	1.86	1.80	1.60	1.65	1.31	1.68	1.15	1.64
32	1.65	1.28	1.65	1.60	1.84	1.76	-	-	-	1.71	1.51	1.81	1.48	1.76	1.32	1.61
33	1.21	1.36	1.83	1.78	2.02	1.88	-	-	-	1.69	-	1.76	1.34	1.78	1.46	1.63
34	1.45	1.45	1.49	-	1.62	1.84	-	-	-	1.95	-	1.54	1.21	1.62	1.37	1.65
35	1.96	-	2.01	-	-	1.92	-	-	-	-	-	1.80	1.56	-	1.44	1.61
36	1.31	-	1.63	-	-	1.23	-	-	-	-	-	1.67	1.44	-	1.70	1.66
37	1.85	-	-	-	-	-	-	-	-	-	-	-	1.16	-	1.67	1.38
38	1.19	-	-	-	-	-	-	-	-	-	-	-	-	-	1.27	-

	Nominal concentration Dexamethasone [µg/L]															
39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean	**1.67**	**1.77**	**1.72**	**1.76**	**1.77**	**1.76**	**2.01**	**1.77**	**1.82**	**1.75**	**1.68**	**1.72**	**1.56**	**1.69**	**1.55**	**1.61**
SD	0.28	0.30	0.20	0.26	0.17	0.17	0.18	0.23	0.15	0.15	0.24	0.17	0.20	0.16	0.18	0.12
RSD	16.8	17.2	11.8	14.5	9.6	9.4	8.7	12.7	8.0	8.3	14.1	9.8	12.7	9.4	11.5	7.2
Total mean				**1.73**				**1.83**				**1.74**				**1.60***
Total SD				0.04				0.12				0.06				0.07
Total RSD				2.6				6.6				3.3				4.2

*statistically significant reduced compared to control, p<0.05, Jonckheere-Terpstra Test; one-sided smaller

C.2.7 First filial generation (F_1), juvenile growth

Table 154: F_1 generation, juvenile growth: survival at day 63 pf

		Nominal concentration Dexamethasone [µg/L]						
		Control	0.32	1.00	3.20	10.0	32.0	100
		Mean measured concentration Dexamethasone [µg/L]						
	Replicate	Control	0.33	0.91	3.19	10.5	34.7	100
Fish numbers after reduction at day 35 pf, [n]	A	20	20	20	20	20	20	20
	B	-(*)	20	20	20	20	20	20
	C	20	20	20	20	20	20	20
	D	20	20	20	20	20	20	20
Survived, day 63 pf [n]	A	20	20	20	20	20	20	20
	B	-(*)	20	20	20	20	20	20
	C	20	20	20	19	20	20	20
	D	20	20	20	20	20	20	20
Survived, day 63 pf [%]	A	100	100	100	100	100	100	100
	B	-(*)	100	100	100	100	100	100
	C	100	100	100	95.0	100	100	100
	D	100	100	100	100	100	100	100
	Mean	**100**	**100**	**100**	**98.8**	**100**	**100**	**100**
	SD	0.0	0.0	0.0	2.5	0.0	0.0	0.0
	RSD	0.0	0.0	0.0	2.5	0.0	0.0	0.0

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

Table 155: F_1 generation, juvenile growth: individual total length at day 63 pf [cm]; control, 0.32 and 1.00 µg Dexamethasone/L (nominal), respective 0.33 and 0.91 µg Dexamethasone/L (mean measured)

	Nominal concentration Dexamethasone [µg/L]											
	Control				0.32				1.00			
	Mean measured concentration Dexamethasone [µg/L]											
	Control				0.33				0.91			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Individual total length 63 pf [cm]											
1	3.02	- (*)	2.96	3.14	2.83	3.28	3.16	2.99	3.23	3.19	3.24	3.30
2	3.16	- (*)	3.10	3.18	3.27	3.19	2.74	3.29	3.25	2.93	2.77	3.09
3	3.22	- (*)	3.26	2.66	2.52	3.01	3.12	3.04	3.06	2.83	3.26	3.04
4	2.93	- (*)	3.04	3.18	3.32	2.98	2.99	3.17	3.00	3.51	3.02	2.73
5	2.94	- (*)	3.35	2.80	3.07	2.78	2.99	2.89	2.95	3.02	3.12	2.75
6	3.15	- (*)	2.77	3.30	2.87	3.50	3.15	3.00	3.16	2.95	3.13	3.12
7	3.19	- (*)	3.14	2.94	3.05	3.16	3.00	3.06	2.75	3.14	3.28	2.88
8	3.12	- (*)	2.91	2.83	2.99	3.10	3.07	3.06	3.02	2.99	3.14	2.96
9	3.21	- (*)	3.16	3.25	3.00	3.13	2.98	3.09	2.74	3.00	3.37	3.16
10	3.02	- (*)	2.80	3.26	2.83	2.95	2.96	2.83	2.85	3.01	3.07	3.26
11	3.06	- (*)	3.28	3.10	3.28	3.04	2.97	2.88	3.08	3.10	2.68	2.71
12	3.08	- (*)	3.04	3.16	3.15	3.30	2.84	3.01	2.97	3.23	3.13	3.17
13	3.24	- (*)	3.02	2.69	2.83	2.93	3.05	2.99	3.42	2.87	2.88	2.97

	Nominal concentration Dexamethasone [µg/L]											
14	3.00	- (*)	3.01	3.03	3.16	2.97	2.94	2.57	3.16	2.64	3.05	2.99
15	3.10	- (*)	3.11	2.89	3.20	3.01	2.90	2.85	3.39	3.19	3.31	3.23
16	3.00	- (*)	2.96	2.83	3.25	3.38	2.97	3.24	3.09	3.33	3.21	3.33
17	3.05	- (*)	2.88	2.99	2.75	3.04	2.86	3.31	3.13	2.97	3.24	3.15
18	3.07	- (*)	2.82	3.43	3.15	3.25	2.99	3.14	3.07	3.48	3.14	2.81
19	2.79	- (*)	3.16	2.84	3.47	2.99	2.94	3.17	2.94	3.20	3.20	2.68
20	2.90	- (*)	3.23	3.03	3.19	3.23	2.76	2.97	3.25	2.87	2.96	3.10
Mean	**3.06**	**- (*)**	**3.05**	**3.03**	**3.06**	**3.11**	**2.97**	**3.03**	**3.08**	**3.07**	**3.11**	**3.02**
SD	0.12	-	0.17	0.21	0.23	0.17	0.11	0.17	0.18	0.22	0.18	0.20
RSD	3.8	-	5.4	7.0	7.6	5.6	3.8	5.8	6.0	7.0	5.7	6.8
Total mean				**3.05**				**3.04**				**3.07**
Total SD				0.02				0.06				0.04
Total RSD				0.6				2.0				1.2

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

Table 156: F_1 generation, juvenile growth: individual total length at day 63 pf [cm]; 3.20, 10.0, 32.0 and 100 µg Dexamethasone/L (nominal), 3.19, 10.5, 34.7 and 100 µg Dexamethsone/L (mean measured)

	Nominal concentration Dexamethasone [µg/L]															
	3.20				10.0				32.0				100			
	Mean measured concentration Dexamethasone [µg/L]															
	3.19				10.5				34.7				100			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Individual total length day 63 pf [cm]															
1	2.92	2.98	2.98	3.07	3.17	2.99	3.11	2.67	2.97	2.97	2.99	2.80	2.49	2.91	2.91	2.60
2	3.03	3.10	2.80	3.08	2.70	2.95	2.99	2.93	3.18	2.79	2.81	2.93	2.44	3.10	2.40	2.66
3	2.91	2.83	2.94	3.14	3.07	3.14	3.13	2.99	3.18	3.20	2.64	2.99	2.43	2.56	2.54	2.64
4	3.28	3.17	2.89	3.08	2.91	3.03	3.10	3.17	2.84	2.84	2.63	2.95	3.12	2.91	2.45	2.88
5	2.80	2.95	2.94	2.85	3.20	3.06	3.40	3.09	3.24	2.94	2.83	3.08	2.66	2.72	2.85	2.72
6	2.56	3.26	2.99	2.98	3.12	3.08	3.35	2.99	2.68	2.98	2.69	2.70	2.44	2.88	2.67	2.61
7	2.59	2.84	2.82	2.91	2.93	2.96	2.97	2.77	3.14	2.81	2.88	2.63	3.08	2.95	2.50	2.84
8	2.90	2.76	3.28	2.89	2.98	2.93	3.21	3.00	2.72	2.94	2.84	2.76	2.49	2.87	2.22	2.64
9	2.99	3.30	3.24	3.28	3.10	2.77	2.95	3.20	2.79	2.99	2.55	2.41	2.88	2.71	2.83	2.43
10	2.93	3.16	3.05	3.36	3.22	2.72	2.95	3.14	3.13	2.93	2.96	2.82	2.79	2.78	2.61	3.05
11	3.10	2.99	2.80	2.75	3.07	3.14	3.24	2.87	3.14	3.04	2.67	3.04	2.86	2.76	2.76	3.00
12	2.34	3.18	3.03	3.46	3.08	3.00	3.24	3.05	2.99	2.79	3.04	2.89	3.06	3.05	2.64	2.56
13	2.99	3.26	3.03	2.72	2.97	2.98	3.30	3.04	2.96	2.96	3.15	2.67	2.92	2.91	2.79	2.79
14	2.79	2.98	3.17	2.67	2.79	2.71	3.05	3.06	2.94	3.12	2.60	2.67	2.93	2.76	2.78	2.81

	Nominal concentration Dexamethasone [µg/L]															
15	3.11	3.04	2.70	3.03	3.02	2.88	3.06	2.69	3.09	2.92	2.88	3.02	2.80	2.74	2.99	2.47
16	2.75	2.97	3.21	3.24	2.94	2.92	3.12	3.07	3.22	2.84	3.13	3.00	2.64	2.57	2.80	2.82
17	3.14	3.17	3.05	2.81	3.12	2.79	3.40	3.01	3.04	2.84	3.04	2.65	2.85	2.97	2.79	2.76
18	3.02	3.52	3.18	3.39	3.14	2.92	3.09	2.92	2.92	2.80	2.86	3.00	2.49	2.67	2.64	2.55
19	2.89	3.45	2.76	3.09	3.27	3.02	3.07	2.89	2.79	2.81	2.92	2.87	2.95	2.58	2.97	2.75
20	2.81	3.15	-	2.85	2.99	2.85	-	2.86	3.27	2.90	3.08	3.01	2.52	2.86	2.55	2.81
21	-	-	-	-	-	-	-	-	-	-	2.97	-	-	-	-	-
Mean	**2.89**	**3.10**	**2.99**	**3.03**	**3.04**	**2.94**	**3.14**	**2.97**	**3.01**	**2.92**	**2.86**	**2.84**	**2.74**	**2.81**	**2.68**	**2.72**
SD	0.22	0.20	0.17	0.23	0.14	0.13	0.14	0.15	0.18	0.11	0.18	0.18	0.24	0.15	0.20	0.16
RSD	7.6	6.4	5.7	7.6	4.7	4.3	4.6	4.9	6.0	3.8	6.3	6.3	8.6	5.5	7.4	6.0
Total mean				**3.01**				**3.02**				**2.91 (*)**				**2.74 (*)**
Total SD				0.09				0.09				0.07				0.05
Total RSD				2.9				3.0				2.6				2.0

(*) Statistically significant reduced compared to control, p<0.05, Williams test, one-sided smaller.

C.2.8 First filial generation (F_1), reproduction

Table 157: F_1 generation, reproduction: fecundity and fertilisation rate; control

	Control																			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Time of exposure [day]	Fertilised eggs [n]				Total egg number [n]				Fertilisation rate [%]				Female number [n]				Total egg number per female [n]			
57	0	-(*)	0	0	0	-(*)	0	0	-	-(*)	-	-	10	-(*)	11	13	0	-(*)	0	0
58	0	-(*)	9	0	0	-(*)	10	0	-	-(*)	90.0	-	10	-(*)	11	13	0	-(*)	1	0
59	0	-(*)	18	18	0	-(*)	18	24	-	-(*)	100	75.0	10	-(*)	11	13	0	-(*)	2	2
60	0	-(*)	38	0	0	-(*)	43	0	-	-(*)	88.4	-	10	-(*)	11	13	0	-(*)	4	0
61	27	-(*)	17	0	35	-(*)	17	0	77.1	-(*)	100	-	10	-(*)	11	13	4	-(*)	2	0
62	0	-(*)	8	36	0	-(*)	8	43	-	-(*)	100	83.7	10	-(*)	11	13	0	-(*)	1	3
63	25	-(*)	21	2	39	-(*)	32	17	64.1	-(*)	65.6	11.8	10	-(*)	11	13	4	-(*)	3	1
64	21	-(*)	3	38	31	-(*)	3	42	67.7	-(*)	100	90.5	10	-(*)	11	13	3	-(*)	0	3
65	27	-(*)	76	105	29	-(*)	88	124	93.1	-(*)	86.4	84.7	10	-(*)	11	13	3	-(*)	8	10
66	62	-(*)	28	78	79	-(*)	38	100	78.5	-(*)	73.7	78.0	10	-(*)	11	13	8	-(*)	3	8
67	122	-(*)	46	9	151	-(*)	51	10	80.8	-(*)	90.2	90.0	10	-(*)	11	13	15	-(*)	5	1
68	26	-(*)	50	132	27	-(*)	62	158	96.3	-(*)	80.6	83.5	10	-(*)	11	13	3	-(*)	6	12
69	273	-(*)	38	114	301	-(*)	39	116	90.7	-(*)	97.4	98.3	10	-(*)	11	13	30	-(*)	4	9
70	52	-(*)	107	122	68	-(*)	122	153	76.5	-(*)	87.7	79.7	10	-(*)	11	13	7	-(*)	11	12
71	144	-(*)	175	70	153	-(*)	189	93	94.1	-(*)	92.6	75.3	10	-(*)	11	13	15	-(*)	17	7

	Control																			
72	191	-(*)	305	311	205	-(*)	351	356	93.2	-(*)	86.9	87.4	10	-(*)	11	13	21	-(*)	32	27
73	71	-(*)	186	157	76	-(*)	210	165	93.4	-(*)	88.6	95.2	10	-(*)	11	13	8	-(*)	19	13
74	237	-(*)	196	277	260	-(*)	235	373	91.2	-(*)	83.4	74.3	10	-(*)	11	13	26	-(*)	21	29
75	115	-(*)	227	190	118	-(*)	252	202	97.5	-(*)	90.1	94.1	10	-(*)	11	13	12	-(*)	23	16
76	272	-(*)	364	366	290	-(*)	414	423	93.8	-(*)	87.9	86.5	10	-(*)	11	13	29	-(*)	38	33
77	168	-(*)	199	293	180	-(*)	258	310	93.3	-(*)	77.1	94.5	10	-(*)	11	13	18	-(*)	23	24
78	222	-(*)	252	335	232	-(*)	302	355	95.7	-(*)	83.4	94.4	10	-(*)	11	13	23	-(*)	27	27
79	250	-(*)	235	295	260	-(*)	260	311	96.2	-(*)	90.4	94.9	10	-(*)	11	13	26	-(*)	24	24
80	203	-(*)	179	502	210	-(*)	192	545	96.7	-(*)	93.2	92.1	10	-(*)	11	13	21	-(*)	17	42
81	218	-(*)	364	372	224	-(*)	385	421	97.3	-(*)	94.5	88.4	10	-(*)	11	13	22	-(*)	35	32
82	110	-(*)	191	328	119	-(*)	204	349	92.4	-(*)	93.6	94.0	10	-(*)	11	13	12	-(*)	19	27
83	155	-(*)	179	319	180	-(*)	217	386	86.1	-(*)	82.5	82.6	10	-(*)	11	13	18	-(*)	20	30
84	79	-(*)	208	359	82	-(*)	242	408	96.3	-(*)	86.0	88.0	10	-(*)	11	13	8	-(*)	22	31
85	186	-(*)	372	404	190	-(*)	391	474	97.9	-(*)	95.1	85.2	10	-(*)	11	13	19	-(*)	36	36
86	142	-(*)	214	505	148	-(*)	222	603	95.9	-(*)	96.4	83.7	10	-(*)	11	13	15	-(*)	20	46
87	409	-(*)	443	376	422	-(*)	458	415	96.9	-(*)	96.7	90.6	10	-(*)	11	13	42	-(*)	42	32
88	184	-(*)	277	573	189	-(*)	286	661	97.4	-(*)	96.9	86.7	10	-(*)	11	13	19	-(*)	26	51
89	518	-(*)	389	439	533	-(*)	398	531	97.2	-(*)	97.7	82.7	10	-(*)	11	13	53	-(*)	36	41
90	93	-(*)	371	550	101	-(*)	386	582	92.1	-(*)	96.1	94.5	10	-(*)	11	13	10	-(*)	35	45
91	227	-(*)	253	325	233	-(*)	289	422	97.4	-(*)	87.5	77.0	10	-(*)	11	13	23	-(*)	26	32

	Control																			
92	188	-(*)	226	432	193	-(*)	276	499	97.4	-(*)	81.9	86.6	10	-(*)	11	13	19	-(*)	25	38
93	209	-(*)	295	562	214	-(*)	305	624	97.7	-(*)	96.7	90.1	10	-(*)	11	13	21	-(*)	28	48
94	204	-(*)	394	736	210	-(*)	410	847	97.1	-(*)	96.1	86.9	10	-(*)	11	13	21	-(*)	37	65
Mean	**-**	**-(*)**	**-**	**-**	**174**	**-(*)**	**230**	**311**	**92.8**	**-(*)**	**88.9**	**88.1**	**-**	**-(*)**	**-**	**-**	**17**	**-(*)**	**21**	**24**
SD	-	-	-	-	77	-	107	158	5.6	-	5.5	6.8	-	-	-	-	8	-	10	12
RSD	-	-	-	-	44.1	-	46.7	50.8	6.1	-	6.2	7.7	-	-	-	-	44.1	-	46.7	50.8
Cumulative egg number [n]					**5782**	**-(*)**	**7663**	**11142**												

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

Green marked table fields: counting period of 20 days, starting with at least 15 eggs and fertility ≥80% in the controls on three consecutive days.

The mean values were calculated concidering the green marked fields, only.

Table 158: F_1 generation, reproduction: fecundity and fertilisation rate; 0.32 µg Dexamethasone/L (nominal), respective 0.33 µg Dexamethasone/L (mean measured)

	Nominal concentration: 0.32 µg Dexamethasone/L Mean measured concentration: 0.33 µg Dexamethasone/L																			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Time of exposure [day]	**Fertilised eggs [n]**				**Total egg number [n]**				**Fertilisation rate [%]**				**Female number [n]**				**Total egg number per female [n]**			
57	0	0	0	7	0	0	0	8	-	-	-	87.5	11	13	13	15	0	0	0	1
58	36	0	0	0	36	0	0	0	100	-	-	-	11	13	13	15	3	0	0	0
59	0	0	17	15	0	0	21	19	-	-	81.0	78.9	11	13	13	15	0	0	2	1
60	17	7	12	0	17	8	14	0	100	87.5	85.7	-	11	13	13	15	2	1	1	0
61	3	0	0	67	4	0	0	69	75.0	-	-	97.1	11	13	13	15	0	0	0	5
62	80	0	25	136	83	0	27	137	96.4	-	92.6	99.3	11	13	13	15	8	0	2	9
63	50	0	60	10	57	0	65	12	87.7	-	92.3	83.3	11	13	13	15	5	0	5	1
64	59	0	2	182	69	0	2	250	85.5	-	100.0	72.8	11	13	13	15	6	0	0	17
65	160	0	39	30	165	0	41	42	97.0	-	95.1	71.4	11	13	13	15	15	0	3	3
66	73	0	22	206	85	0	23	236	85.9	-	95.7	87.3	11	13	13	15	8	0	2	16
67	197	0	60	47	205	0	74	67	96.1	-	81.1	70.1	11	13	13	15	19	0	6	4
68	47	43	121	213	51	47	145	237	92.2	91.5	83.4	89.9	11	13	13	15	5	4	11	16
69	32	0	70	116	32	0	75	121	100	-	93.3	95.9	11	13	13	15	3	0	6	8
70	125	2	110	326	131	2	133	370	95.4	100	82.7	88.1	11	13	13	15	12	0	10	25
71	104	0	102	103	110	0	112	111	94.5	-	91.1	92.8	11	13	13	15	10	0	9	7

	Nominal concentration: 0.32 µg Dexamethasone/L **Mean measured concentration: 0.33 µg Dexamethasone/L**																			
72	124	50	305	606	131	51	412	648	94.7	98.0	74.0	93.5	11	13	13	15	12	4	32	43
73	367	51	235	176	372	51	247	197	98.7	100	95.1	89.3	11	13	13	15	34	4	19	13
74	82	39	307	502	92	42	393	606	89.1	92.9	78.1	82.8	11	13	13	15	8	3	30	40
75	249	14	197	135	256	15	223	139	97.3	93.3	88.3	97.1	11	13	13	15	23	1	17	9
76	184	70	436	376	227	71	533	444	81.1	98.6	81.8	84.7	11	13	13	15	21	5	41	30
77	62	258	185	304	64	261	195	312	96.9	98.9	94.9	97.4	11	13	13	15	6	20	15	21
78	100	90	330	274	105	91	355	282	95.2	98.9	93.0	97.2	11	13	13	15	10	7	27	19
79	193	54	227	300	203	55	247	310	95.1	98.2	91.9	96.8	11	13	13	15	18	4	19	21
80	295	32	532	511	307	32	549	527	96.1	100	96.9	97.0	11	13	13	15	28	2	42	35
81	155	511	253	353	167	524	262	369	92.8	97.5	96.6	95.7	11	13	13	15	15	40	20	25
82	290	40	508	652	303	41	530	705	95.7	97.6	95.8	92.5	11	13	13	15	28	3	41	47
83	132	169	213	357	186	186	237	368	71.0	90.9	89.9	97.0	11	13	13	15	17	14	18	25
84	360	136	286	532	381	140	297	553	94.5	97.1	96.3	96.2	11	13	13	15	35	11	23	37
85	109	197	350	375	122	205	362	390	89.3	96.1	96.7	96.2	11	13	13	15	11	16	28	26
86	189	89	544	682	198	90	576	717	95.5	98.9	94.4	95.1	11	13	13	15	18	7	44	48
87	134	0	305	490	135	0	311	508	99.3	-	98.1	96.5	11	13	13	15	12	0	24	34
88	202	105	369	353	209	106	382	365	96.7	99.1	96.6	96.7	11	13	13	15	19	8	29	24
89	109	233	471	508	114	234	487	542	95.6	99.6	96.7	93.7	11	13	13	15	10	18	37	36
90	148	480	283	209	151	490	285	213	98.0	98.0	99.3	98.1	11	13	13	15	14	38	22	14
91	137	314	446	476	147	323	462	493	93.2	97.2	96.5	96.6	11	13	13	15	13	25	36	33

	Nominal concentration: 0.32 µg Dexamethasone/L **Mean measured concentration: 0.33 µg Dexamethasone/L**																			
92	95	135	183	342	98	138	189	354	96.9	97.8	96.8	96.6	11	13	13	15	9	11	15	24
93	56	288	405	481	56	293	421	498	100	98.3	96.2	96.6	11	13	13	15	5	23	32	33
94	123	27	550	364	124	31	565	377	99.2	87.1	97.3	96.6	11	13	13	15	11	2	43	25
Mean	**-**	**-**	**-**	**-**	**146**	**150**	**254**	**396**	**93.4**	**97.4**	**89.8**	**93.6**	**-**	**-**	**-**	**-**	**13**	**12**	**20**	**26**
SD	-	-	-	-	92	151	164	189	5.0	2.5	7.0	4.4	-	-	-	-	8	12	13	13
RSD	-	-	-	-	63.0	100.2	64.6	47.8	5.3	2.6	7.8	4.7	-	-	-	-	63.0	100.2	64.6	47.8
Cumulative egg number [n]					**5193**	**3527**	**9252**	**11596**												

Green marked table fields: counting period of 20 days, starting with at least 15 eggs and fertility ≥80% in the controls on three consecutive days

The mean values were calculated concidering the green marked fields, only.

Table 159: F_1 generation, reproduction: fecundity and fertilisation rate; 1.00 µg Dexamethasone/L (nominal), respective 0.91 µg Dexamethasone/L (mean measured)

	Nominal concentration: 1.00 µg Dexamethasone/L Mean measured concentration: 0.91 µg Dexamethasone/L																			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Time of exposure [day]	**Fertilised eggs [n]**				**Total egg number [n]**				**Fertilisation rate [%]**				**Female number [n]**				**Total egg number per female [n]**			
57	0	0	0	0	0	0	0	0	-	-	-	-	17	16	10	13	0	0	0	0
58	0	0	0	0	0	0	0	0	-	-	-	-	17	16	10	13	0	0	0	0
59	0	0	0	0	0	0	0	0	-	-	-	-	17	16	10	13	0	0	0	0
60	0	0	0	0	0	0	0	0	-	-	-	-	17	16	10	13	0	0	0	0
61	0	0	29	0	0	0	31	0	-	-	93.5	-	17	16	10	13	0	0	3	0
62	0	0	0	0	0	0	0	0	-	-	-	-	17	16	10	13	0	0	0	0
63	0	0	50	0	0	0	55	0	-	-	90.9	-	17	16	10	13	0	0	6	0
64	0	0	0	0	0	0	0	0	-	-	-	-	17	16	10	13	0	0	0	0
65	0	0	87	0	0	0	103	0	-	-	84.5	-	17	16	10	13	0	0	10	0
66	4	0	41	0	4	0	43	0	100	-	95.3	-	17	16	10	13	0	0	4	0
67	26	0	114	0	26	0	137	0	100	-	83.2	-	17	16	10	13	2	0	14	0
68	22	1	81	2	32	4	86	3	68.8	25.0	94.2	66.7	17	16	10	13	2	0	9	0
69	69	13	127	0	72	13	139	0	95.8	100	91.4	-	17	16	10	13	4	1	14	0
70	273	63	176	32	291	68	209	50	93.8	92.6	84.2	64.0	17	16	10	13	17	4	21	4
71	134	116	105	65	140	152	114	68	95.7	76.3	92.1	95.6	17	16	10	13	8	10	11	5

	Nominal concentration: 1.00 µg Dexamethasone/L **Mean measured concentration: 0.91 µg Dexamethasone/L**																			
72	271	105	165	83	287	113	187	99	94.4	92.9	88.2	83.8	17	16	10	13	17	7	19	8
73	224	127	217	179	227	138	222	191	98.7	92.0	97.7	93.7	17	16	10	13	13	9	22	15
74	288	178	90	185	301	242	112	228	95.7	73.6	80.4	81.1	17	16	10	13	18	15	11	18
75	245	135	307	74	251	144	331	78	97.6	93.8	92.7	94.9	17	16	10	13	15	9	33	6
76	465	196	217	216	496	230	280	248	93.8	85.2	77.5	87.1	17	16	10	13	29	14	28	19
77	223	333	285	277	230	360	301	284	97.0	92.5	94.7	97.5	17	16	10	13	14	23	30	22
78	365	250	222	280	390	270	234	288	93.6	92.6	94.9	97.2	17	16	10	13	23	17	23	22
79	299	335	189	200	319	357	199	205	93.7	93.8	95.0	97.6	17	16	10	13	19	22	20	16
80	556	604	265	175	570	679	274	182	97.5	89.0	96.7	96.2	17	16	10	13	34	42	27	14
81	364	307	317	405	369	358	339	435	98.6	85.8	93.5	93.1	17	16	10	13	22	22	34	33
82	375	323	191	333	399	336	212	360	94.0	96.1	90.1	92.5	17	16	10	13	23	21	21	28
83	136	204	113	268	161	240	142	403	84.5	85.0	79.6	66.5	17	16	10	13	9	15	14	31
84	311	513	366	304	326	584	437	394	95.4	87.8	83.8	77.2	17	16	10	13	19	37	44	30
85	434	409	110	400	444	440	121	461	97.7	93.0	90.9	86.8	17	16	10	13	26	28	12	35
86	703	626	682	354	747	692	714	415	94.1	90.5	95.5	85.3	17	16	10	13	44	43	71	32
87	286	194	15	270	292	207	16	283	97.9	93.7	93.8	95.4	17	16	10	13	17	13	2	22
88	558	640	426	546	571	744	458	599	97.7	86.0	93.0	91.2	17	16	10	13	34	47	46	46
89	157	177	136	440	177	205	150	648	88.7	86.3	90.7	67.9	17	16	10	13	10	13	15	50
90	414	804	362	455	431	871	407	509	96.1	92.3	88.9	89.4	17	16	10	13	25	54	41	39
91	158	277	327	176	177	304	424	187	89.3	91.1	77.1	94.1	17	16	10	13	10	19	42	14

	Nominal concentration: 1.00 µg Dexamethasone/L **Mean measured concentration: 0.91 µg Dexamethasone/L**																			
92	382	196	312	371	392	210	351	432	97.4	93.3	88.9	85.9	17	16	10	13	23	13	35	33
93	627	413	381	565	653	437	404	621	96.0	94.5	94.3	91.0	17	16	10	13	38	27	40	48
94	543	511	382	326	557	646	415	365	97.5	79.1	92.0	89.3	17	16	10	13	33	40	42	28
Mean	**-**	**-**	**-**	**-**	**344**	**416**	**205**	**319**	**95.4**	**90.1**	**89.5**	**88.5**	**-**	**-**	**-**	**-**	**20**	**26**	**21**	**25**
SD	-	-	-	-	162	213	100	165	3.1	4.4	6.3	9.3	-	-	-	-	10	13	10	13
RSD	-	-	-	-	47.1	51.3	48.7	51.6	3.3	4.8	7.0	10.5	-	-	-	-	47.1	51.3	48.7	51.6
Cumulative egg number [n]					**9332**	**9044**	**7647**	**8036**												

Green marked table fields: counting period of 20 days, starting with at least 15 eggs and fertility ≥80% in the controls on three consecutive days

The mean values were calculated concidering the green marked fields, only.

Table 160: F_1 generation, reproduction: fecundity and fertilisation rate; 3.20 µg Dexamethasone/L (nominal), respective 3.19 µg Dexamethasone/L (mean measured)

	Nominal concentration: 3.20 µg Dexamethasone/L Mean measured concentration: 3.19 µg Dexamethasone/L																			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Time of exposure [day]	**Fertilised eggs [n]**				**Total egg number [n]**				**Fertilisation rate [%]**				**Female number [n]**				**Total egg number per female [n]**			
57	0	109	0	0	0	117	0	0	-	93.2	-	-	15	11	6	16	0	11	0	0
58	0	16	25	0	0	29	27	0	-	55.2	92.6	-	15	11	6	16	0	3	5	0
59	0	77	0	0	0	83	0	0	-	92.8	-	-	15	11	6	16	0	8	0	0
60	0	0	57	0	0	0	58	0	-	-	98.3	-	15	11	6	16	0	0	10	0
61	0	202	38	0	0	227	42	0	-	89.0	90.5	-	15	11	6	16	0	21	7	0
62	0	37	0	0	0	38	0	0	-	97.4	-	-	15	11	6	16	0	3	0	0
63	0	137	16	0	0	163	19	0	-	84.0	84.2	-	15	11	6	16	0	15	3	0
64	0	63	0	0	0	66	0	0	-	95.5	-	-	15	11	6	16	0	6	0	0
65	4	60	0	0	8	65	0	0	50.0	92.3	-	-	15	11	6	16	1	6	0	0
66	0	63	9	0	0	72	12	0	-	87.5	75.0	-	15	11	6	16	0	7	2	0
67	0	157	84	0	0	173	87	0	-	90.8	96.6	-	15	11	6	16	0	16	15	0
68	71	126	97	0	80	131	107	0	88.8	96.2	90.7	-	15	11	6	16	5	12	18	0
69	0	154	121	0	0	157	136	0	-	98.1	89.0	-	15	11	6	16	0	14	23	0
70	77	395	69	0	103	415	74	0	74.8	95.2	93.2	-	15	11	6	16	7	38	12	0
71	21	126	169	66	29	128	172	80	72.4	98.4	98.3	82.5	15	11	6	16	2	12	29	5

	Nominal concentration: 3.20 µg Dexamethasone/L **Mean measured concentration: 3.19 µg Dexamethasone/L**																			
72	162	123	171	84	180	123	180	138	90.0	100	95.0	60.9	15	11	6	16	12	11	30	9
73	45	212	145	165	47	218	153	202	95.7	97.2	94.8	81.7	15	11	6	16	3	20	26	13
74	273	320	58	85	328	345	64	122	83.2	92.8	90.6	69.7	15	11	6	16	22	31	11	8
75	31	181	182	223	36	185	186	275	86.1	97.8	97.8	81.1	15	11	6	16	2	17	31	17
76	120	293	88	88	208	306	104	165	57.7	95.8	84.6	53.3	15	11	6	16	14	28	17	10
77	365	230	124	174	408	236	125	245	89.5	97.5	99.2	71.0	15	11	6	16	27	21	21	15
78	199	251	100	175	234	258	102	240	85.0	97.3	98.0	72.9	15	11	6	16	16	23	17	15
79	244	211	122	230	266	219	123	296	91.7	96.3	99.2	77.7	15	11	6	16	18	20	21	19
80	171	85	195	432	182	86	197	541	94.0	98.8	99.0	79.9	15	11	6	16	12	8	33	34
81	443	271	247	123	475	272	257	126	93.3	99.6	96.1	97.6	15	11	6	16	32	25	43	8
82	374	138	155	256	409	142	162	272	91.4	97.2	95.7	94.1	15	11	6	16	27	13	27	17
83	536	228	210	317	646	231	219	395	83.0	98.7	95.9	80.3	15	11	6	16	43	21	37	25
84	218	119	100	257	335	120	103	282	65.1	99.2	97.1	91.1	15	11	6	16	22	11	17	18
85	709	322	216	686	771	332	220	789	92.0	97.0	98.2	86.9	15	11	6	16	51	30	37	49
86	431	288	172	476	552	292	184	598	78.1	98.6	93.5	79.6	15	11	6	16	37	27	31	37
87	264	235	202	188	289	241	206	198	91.3	97.5	98.1	94.9	15	11	6	16	19	22	34	12
88	649	176	170	470	744	180	179	516	87.2	97.8	95.0	91.1	15	11	6	16	50	16	30	32
89	500	225	209	451	600	228	212	565	83.3	98.7	98.6	79.8	15	11	6	16	40	21	35	35
90	417	196	196	393	449	210	205	425	92.9	93.3	95.6	92.5	15	11	6	16	30	19	34	27
91	362	397	247	487	373	404	263	528	97.1	98.3	93.9	92.2	15	11	6	16	25	37	44	33

	Nominal concentration: 3.20 µg Dexamethasone/L **Mean measured concentration: 3.19 µg Dexamethasone/L**																			
92	272	51	117	460	280	53	118	496	97.1	96.2	99.2	92.7	15	11	6	16	19	5	20	31
93	559	398	178	502	593	406	194	568	94.3	98.0	91.8	88.4	15	11	6	16	40	37	32	36
94	418	72	163	692	446	72	165	762	93.7	100	98.8	90.8	15	11	6	16	30	7	28	48
Mean	**-**	**-**	**-**	**-**	**377**	**181**	**148**	**414**	**86.4**	**94.9**	**95.1**	**84.4**	**-**	**-**	**-**	**-**	**25**	**16**	**25**	**26**
SD	-	-	-	-	210	100	53	193	9.9	4.2	3.9	10.6	-	-	-	-	14	9	9	12
RSD	-	-	-	-	55.7	55.2	36.2	46.7	11.5	4.4	4.1	12.6	-	-	-	-	55.7	55.2	36.2	46.7
Cumulative egg number [n]					**9071**	**7023**	**4655**	**8824**												

Green marked table fields: counting period of 20 days, starting with at least 15 eggs and fertility ≥80% in the controls on three consecutive days

The mean values were calculated concidering the green marked fields, only.

Table 161: F_1 generation, reproduction: fecundity and fertilisation rate; 10.0 µg Dexamethasone/L (nominal), respective 10.5 µg Dexamethasone/L (mean measured)

	Nominal concentration: Mean measured concentration:				10.0 µg Dexamethasone/L 10.5 µg Dexamethasone/L															
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Time of exposure [day]	**Fertilised eggs [n]**				**Total egg number [n]**				**Fertilisation rate [%]**				**Female number [n]**				**Total egg number per female [n]**			
57	63	0	36	96	65	0	40	100	96.9	-	90.0	96.0	9	8	10	12	7	0	4	8
58	0	56	95	0	0	59	99	0	-	94.9	96.0	-	9	8	10	12	0	7	10	0
59	120	0	6	117	127	0	7	126	94.5	-	85.7	92.9	9	8	10	12	14	0	1	11
60	0	72	10	42	0	73	10	50	-	98.6	100	84.0	9	8	10	12	0	9	1	4
61	2	0	48	216	2	0	59	229	100	-	81.4	94.3	9	8	10	12	0	0	6	19
62	0	97	37	88	0	102	44	93	-	95.1	84.1	94.6	9	8	10	12	0	13	4	8
63	49	0	22	213	56	0	25	234	87.5	-	88.0	91.0	9	8	10	12	6	0	3	20
64	72	89	142	99	77	94	149	121	93.5	94.7	95.3	81.8	9	8	10	12	9	12	15	10
65	51	0	89	332	55	0	94	368	92.7	-	94.7	90.2	9	8	10	12	6	0	9	31
66	67	64	167	96	73	70	171	109	91.8	91.4	97.7	88.1	9	8	10	12	8	9	17	9
67	18	78	56	350	18	85	67	385	100	91.8	83.6	90.9	9	8	10	12	2	11	7	32
68	224	135	344	216	234	140	362	254	95.7	96.4	95.0	85.0	9	8	10	12	26	18	36	21
69	44	0	98	424	45	0	105	443	97.8	-	93.3	95.7	9	8	10	12	5	0	11	37
70	110	191	210	146	130	204	239	166	84.6	93.6	87.9	88.0	9	8	10	12	14	26	24	14

	Nominal concentration: **Mean measured concentration:**				**10.0 µg Dexamethasone/L** **10.5 µg Dexamethasone/L**																			
71	79	182	131	441	119	202	138	470	66.4	90.1	94.9	93.8	9	8	10	12	13	25	14	39
72	251	95	236	339	283	107	253	357	88.7	88.8	93.3	95.0	9	8	10	12	31	13	25	30
73	121	203	289	529	125	212	306	556	96.8	95.8	94.4	95.1	9	8	10	12	14	27	31	46
74	254	44	319	279	278	45	327	302	91.4	97.8	97.6	92.4	9	8	10	12	31	6	33	25
75	285	297	438	708	307	317	464	773	92.8	93.7	94.4	91.6	9	8	10	12	34	40	46	64
76	522	183	300	339	550	205	315	386	94.9	89.3	95.2	87.8	9	8	10	12	61	26	32	32
77	84	121	341	141	102	149	356	145	82.4	81.2	95.8	97.2	9	8	10	12	11	19	36	12
78	388	152	300	425	400	172	315	452	97.0	88.4	95.2	94.0	9	8	10	12	44	22	32	38
79	232	252	300	188	242	267	317	213	95.9	94.4	94.6	88.3	9	8	10	12	27	33	32	18
80	197	380	288	217	205	397	296	226	96.1	95.7	97.3	96.0	9	8	10	12	23	50	30	19
81	315	240	412	570	337	249	439	594	93.5	96.4	93.8	96.0	9	8	10	12	37	31	44	50
82	334	148	293	462	351	160	305	495	95.2	92.5	96.1	93.3	9	8	10	12	39	20	31	41
83	249	238	425	169	291	256	473	180	85.6	93.0	89.9	93.9	9	8	10	12	32	32	47	15
84	204	286	231	267	254	301	246	281	80.3	95.0	93.9	95.0	9	8	10	12	28	38	25	23
85	247	161	350	305	306	167	365	315	80.7	96.4	95.9	96.8	9	8	10	12	34	21	37	26
86	314	238	462	418	436	275	495	435	72.0	86.5	93.3	96.1	9	8	10	12	48	34	50	36
87	133	217	314	270	137	222	321	283	97.1	97.7	97.8	95.4	9	8	10	12	15	28	32	24
88	398	238	387	314	503	253	400	345	79.1	94.1	96.8	91.0	9	8	10	12	56	32	40	29
89	336	382	452	540	352	431	475	591	95.5	88.6	95.2	91.4	9	8	10	12	39	54	48	49

	Nominal concentration: **Mean measured concentration:**				**10.0 µg Dexamethasone/L** **10.5 µg Dexamethasone/L**															
90	40	158	277	496	40	176	302	514	100	89.8	91.7	96.5	9	8	10	12	4	22	30	43
91	180	172	416	364	286	283	464	415	62.9	60.8	89.7	87.7	9	8	10	12	32	35	46	35
92	209	133	85	196	218	164	88	209	95.9	81.1	96.6	93.8	9	8	10	12	24	21	9	17
93	308	200	475	261	328	284	489	271	93.9	70.4	97.1	96.3	9	8	10	12	36	36	49	23
94	507	192	466	471	519	236	495	494	97.7	81.4	94.1	95.3	9	8	10	12	58	30	50	41
Mean	**-**	**-**	**-**	**-**	**199**	**185**	**220**	**301**	**91.7**	**92.7**	**92.7**	**91.2**	**-**	**-**	**-**	**-**	**22**	**23**	**22**	**25**
SD	-	-	-	-	143	98	128	184	7.4	4.0	4.9	4.2	-	-	-	-	16	12	13	15
RSD	-	-	-	-	71.7	52.7	58.3	61.1	8.1	4.3	5.3	4.6	-	-	-	-	71.7	52.7	58.3	61.1
Cumulative egg number [n]					**7851**	**6357**	**9915**	**11980**												

Green marked table fields: Counting period of 20 days, starting with at least 15 eggs and fertility ≥80% in the controls on three consecutive days.

The mean values were calculated concidering the green marked fields, only.

Table 162: F_1 generation, reproduction: fecundity and fertilisation rate; 32.0 µg Dexamethasone/L (nominal), respective 34.7 µg Dexamethasone/L (mean measured)

	Nominal concentration: 32.0 µg Dexamethasone/L Mean measured concentration: 34.7 µg Dexamethasone/L																			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Time of exposure [day]	Fertilised eggs [n]				Total egg number [n]				Fertilisation rate [%]				Female number [n]				Total egg number per female [n]			
57	0	0	0	0	0	0	0	0	--	-	-	-	17	13	10	16	0	0	0	0
58	0	53	38	0	0	62	43	0	-	85.5	88.4	-	17	13	10	16	0	5	4	0
59	0	22	0	0	0	24	0	0	-	91.7	-	-	17	13	10	16	0	2	0	0
60	0	75	0	50	0	78	0	54	-	96.2	-	92.6	17	13	10	16	0	6	0	3
61	47	68	27	0	47	70	32	0	100	97.1	84.4	-	17	13	10	16	3	5	3	0
62	0	152	0	78	0	160	0	80	-	95.0	-	97.5	17	13	10	16	0	12	0	5
63	169	38	2	102	187	41	3	117	90.4	92.7	66.7	87.2	17	13	10	16	11	3	0	7
64	47	158	36	56	51	164	41	64	92.2	96.3	87.8	87.5	17	13	10	16	3	13	4	4
65	119	52	29	119	134	55	29	132	88.8	94.5	100.0	90.2	17	13	10	16	8	4	3	8
66	145	87	75	15	148	89	80	16	98.0	97.8	93.8	93.8	17	13	10	16	9	7	8	1
67	141	4	95	293	153	9	97	315	92.2	44.4	97.9	93.0	17	13	10	16	9	1	10	20
68	207	187	146	164	230	204	155	176	90.0	91.7	94.2	93.2	17	13	10	16	14	16	16	11
69	107	83	167	251	114	87	169	268	93.9	95.4	98.8	93.7	17	13	10	16	7	7	17	17
70	409	140	158	430	442	154	165	467	92.5	90.9	95.8	92.1	17	13	10	16	26	12	17	29
71	260	303	150	192	266	348	157	218	97.7	87.1	95.5	88.1	17	13	10	16	16	27	16	14

	Nominal concentration: 32.0 µg Dexamethasone/L **Mean measured concentration: 34.7 µg Dexamethasone/L**																			
72	455	279	82	333	478	296	87	342	95.2	94.3	94.3	97.4	17	13	10	16	28	23	9	21
73	512	81	220	594	523	89	228	607	97.9	91.0	96.5	97.9	17	13	10	16	31	7	23	38
74	575	105	145	285	681	113	152	334	84.4	92.9	95.4	85.3	17	13	10	16	40	9	15	21
75	439	156	185	434	486	173	191	485	90.3	90.2	96.9	89.5	17	13	10	16	29	13	19	30
76	353	232	394	545	382	242	408	683	92.4	95.9	96.6	79.8	17	13	10	16	22	19	41	43
77	626	84	201	353	651	86	213	376	96.2	97.7	94.4	93.9	17	13	10	16	38	7	21	24
78	368	112	350	422	395	117	370	445	93.2	95.7	94.6	94.8	17	13	10	16	23	9	37	28
79	425	189	222	350	455	199	237	375	93.4	95.0	93.7	93.3	17	13	10	16	27	15	24	23
80	238	99	238	668	245	101	246	713	97.1	98.0	96.7	93.7	17	13	10	16	14	8	25	45
81	680	228	232	495	705	239	235	518	96.5	95.4	98.7	95.6	17	13	10	16	41	18	24	32
82	320	22	162	253	334	23	173	277	95.8	95.7	93.6	91.3	17	13	10	16	20	2	17	17
83	407	367	230	617	430	391	239	742	94.7	93.9	96.2	83.2	17	13	10	16	25	30	24	46
84	459	147	174	497	479	152	180	542	95.8	96.7	96.7	91.7	17	13	10	16	28	12	18	34
85	590	87	176	366	615	94	179	389	95.9	92.6	98.3	94.1	17	13	10	16	36	7	18	24
86	632	218	242	556	676	232	255	603	93.5	94.0	94.9	92.2	17	13	10	16	40	18	26	38
87	259	186	253	526	263	190	260	543	98.5	97.9	97.3	96.9	17	13	10	16	15	15	26	34
88	656	440	260	492	698	479	277	539	94.0	91.9	93.9	91.3	17	13	10	16	41	37	28	34
89	193	179	250	566	210	190	257	644	91.9	94.2	97.3	87.9	17	13	10	16	12	15	26	40
90	739	127	352	553	773	130	362	590	95.6	97.7	97.2	93.7	17	13	10	16	45	10	36	37
91	573	96	2	577	596	114	2	617	96.1	84.2	100	93.5	17	13	10	16	35	9	0	39

	Nominal concentration: 32.0 µg Dexamethasone/L **Mean measured concentration: 34.7 µg Dexamethasone/L**																			
92	567	187	365	168	600	196	374	208	94.5	95.4	97.6	80.8	17	13	10	16	35	15	37	13
93	222	397	247	655	224	421	261	715	99.1	94.3	94.6	91.6	17	13	10	16	13	32	26	45
94	891	80	135	350	982	85	143	387	90.7	94.1	94.4	90.4	17	13	10	16	58	7	14	24
Mean	**-**	**-**	**-**	**-**	**353**	**127**	**184**	**337**	**93.4**	**90.9**	**95.6**	**91.9**	**21**	**10**	**18**	**21**	**21**	**10**	**18**	**21**
SD	-	-	-	-	198	114	117	205	3.5	11.4	2.6	4.5	12	7	10	13	12	7	10	13
RSD	-	-	-	-	56.1	89.3	63.8	61.0	3.8	12.6	2.7	4.9	56.1	71.1	52.3	61.0	56.1	71.1	52.3	61.0
Cumulative egg number [n]					**13653**	**5897**	**6300**	**13194**												

Green marked table fields: Counting period of 20 days, starting with at least 15 eggs and fertility ≥80% in the controls on three consecutive days.

The mean values were calculated concidering the green marked fields, only.

Table 163: F_1 generation, reproduction: fecundity and fertilisation rate; 100 µg Dexamethasone/L (nominal), respective 100 µg Dexamethasone/L (mean measured)

	Nominal concentration: 100 µg Dexamethasone/L Mean measured concentration: 100 µg Dexamethasone/L																			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Time of exposure [day]	Fertilised eggs [n]				Total egg number [n]				Fertilisation rate [%]				Female number [n]				Total egg number per female [n]			
57	0	0	0	28	0	15	0	0	0	2	-	82.4	8	17	14	15	0	0	0	2
58	0	0	0	0	0	15	0	0	0	0	-	-	8	17	14	15	0	0	0	0
59	0	0	0	50	0	15	0	0	0	4	-	89.3	8	17	14	15	0	0	0	4
60	0	0	0	26	0	15	0	0	0	2	-	86.7	8	17	14	15	0	0	0	2
61	0	0	0	64	0	15	0	0	1	5	0.0	90.1	8	17	14	15	0	0	1	4
62	13	0	13	26	16	15	2	0	1	3	86.7	52.0	8	17	14	15	2	0	1	3
63	0	0	6	45	0	15	0	0	1	6	75.0	46.9	8	17	14	15	0	0	1	6
64	18	0	22	42	25	15	3	0	2	3	95.7	91.3	8	17	14	15	3	0	2	3
65	26	0	58	113	30	15	4	0	4	8	95.1	89.0	8	17	14	15	4	0	4	8
66	20	0	37	82	21	15	3	0	3	6	88.1	90.1	8	17	14	15	3	0	3	6
67	97	0	27	120	105	15	13	0	2	9	100	87.6	8	17	14	15	13	0	2	9
68	0	0	46	72	0	15	0	0	3	5	100	90.0	8	17	14	15	0	0	3	5
69	33	18	17	209	34	15	4	2	1	15	100	92.5	8	17	14	15	4	2	1	14
70	99	0	165	190	102	15	13	0	12	17	95.4	75.7	8	17	14	15	13	0	12	16
71	70	9	157	117	76	15	10	1	11	9	99.4	91.4	8	17	14	15	10	1	11	8

	Nominal concentration: 100 µg Dexamethasone/L **Mean measured concentration: 100 µg Dexamethasone/L**																			
72	51	69	217	204	57	15	7	6	17	17	92.7	82.3	8	17	14	15	7	6	17	16
73	191	48	174	182	201	15	25	5	14	13	91.6	94.8	8	17	14	15	25	5	14	12
74	162	140	142	347	180	15	23	13	11	25	94.7	92.3	8	17	14	15	23	13	11	24
75	217	58	203	167	226	15	28	5	16	13	88.3	87.9	8	17	14	15	28	5	16	12
76	277	68	394	507	297	15	37	8	33	37	84.9	90.7	8	17	14	15	37	8	33	35
77	214	206	262	413	262	15	33	16	20	31	92.3	88.8	8	17	14	15	33	16	20	29
78	225	78	288	353	255	15	32	9	22	27	92.0	88.0	8	17	14	15	32	9	22	25
79	300	206	311	388	330	15	41	15	25	29	90.7	90.7	8	17	14	15	41	15	25	27
80	458	430	371	406	469	15	59	26	29	30	90.5	88.8	8	17	14	15	59	26	29	29
81	265	69	366	515	267	15	33	5	28	37	92.4	93.8	8	17	14	15	33	5	28	34
82	39	308	137	311	40	15	5	21	10	22	93.8	95.4	8	17	14	15	5	21	10	20
83	547	59	580	382	587	15	73	4	44	27	93.2	95.7	8	17	14	15	73	4	44	25
84	104	480	159	307	113	15	14	31	12	21	92.4	95.9	8	17	14	15	14	31	12	20
85	360	167	576	523	382	15	48	11	45	38	92.5	92.9	8	17	14	15	48	11	45	35
86	341	683	582	475	392	15	49	44	43	33	97.2	95.2	8	17	14	15	49	44	43	31
87	243	284	186	464	250	15	31	17	14	33	96.9	94.5	8	17	14	15	31	17	14	31
88	213	950	225	841	219	15	27	64	17	63	93.0	89.5	8	17	14	15	27	64	17	59
89	287	440	764	570	294	15	37	32	55	51	99.3	75.1	8	17	14	15	37	32	55	47
90	227	910	604	757	231	15	29	56	45	54	95.0	93.5	8	17	14	15	29	56	45	51
91	250	555	599	600	260	15	33	35	44	44	96.8	90.8	8	17	14	15	33	35	44	41

	Nominal concentration: 100 µg Dexamethasone/L **Mean measured concentration: 100 µg Dexamethasone/L**																			
92	221	423	209	793	224	15	28	27	15	58	98.1	91.5	8	17	14	15	28	27	15	54
93	271	397	208	362	277	15	35	26	15	27	96.3	91.0	8	17	14	15	35	26	15	25
94	483	551	532	926	514	15	64	35	40	74	96.2	83.7	8	17	14	15	64	35	40	69
Mean	**-**	**-**	**-**	**-**	**183**	**415**	**216**	**284**	**92.9**	**83.9**	**93.5**	**89.8**	**-**	**-**	**-**	**-**	**23**	**24**	**15**	**19**
SD	-	-	-	-	157	287	168	162	4.4	13.7	4.2	4.6	-	-	-	-	20	17	12	11
RSD	-	-	-	-	86.2	69.3	77.7	56.9	4.8	16.4	4.5	5.1	-	-	-	-	86.2	69.3	77.7	56.9
Cumulative egg number [n]					**6736**	**8748**	**9204**	**13427**												

Green marked table fields: Counting period of 20 days, starting with at least 15 eggs and fertility ≥80% in the controls on three consecutive days.

The mean values were calculated concidering the green marked fields, only.

Table 164: F_1 generation, summary: reproduction

		Nominal concentration Dexamethasone [µg/L]						
		control	0.32	1.00	3.20	10.0	32.0	100
		Mean measured concentration Dexamethasone [µg/L]						
	Replicate	Control	0.33	0.91	3.19	10.5	34.7	100
Total eggs per day and female [n]	A	17	13	20	25	22	21	23
	B	-(*)	12	26	16	23	10	24
	C	21	20	21	25	22	18	15
	D	24	26	25	26	25	21	19
	Mean	**21**	**18**	**23**	**23**	**23**	**17**	**20**
	SD	3	7	3	4	1	5	4
	RSD	15.7	38.1	12.6	19.2	6.1	30.2	19.7
Fertilisation rate [%]	A	92.8	93.4	95.4	86.4	91.7	93.4	92.9
	B	-(*)	97.4	90.1	94.9	92.7	90.9	83.9
	C	88.9	89.8	89.5	95.1	92.7	95.6	93.5
	D	88.1	93.6	88.5	84.4	91.2	91.9	89.8
	Mean	**89.9**	**93.5**	**90.9**	**90.2**	**92.1**	**92.9**	**90.0**
	SD	2.5	3.1	3.1	5.6	0.8	2.0	4.4
	RSD	2.8	3.3	3.4	6.2	0.8	2.2	4.9

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

C.2.9 First filial generation (F_1), adult stage

Table 165: F_1 generation, adult stage: survival

		Nominal concentration Dexamethasone [µg/L]						
		Control	**0.32**	**1.00**	**3.20**	**10.0**	**32.0**	**100**
		Mean measured concentration Dexamethasone [µg/L]						
	Replicate	**Control**	**0.33**	**0.91**	**3.19**	**10.5**	**34.7**	**100**
Fish number, day 63 pf [n]	A	20	20	20	20	20	20	20
	B	-(*)	20	20	20	20	20	20
	C	20	20	20	19	20	20	20
	D	20	20	20	20	20	20	20
Survival, adult stage [n]	A	20	20	20	19	19	20	18
	B	-(*)	20	20	20	20	19	19
	C	20	20	19	19	19	20	20
	D	20	20	20	20	20	20	20
Survival, adult stage [%]	A	100	100	100	95.0	95.0	100	90.0
	B	-(*)	100	100	100	100	95.0	95.0
	C	100	100	95.0	100	95.0	100	100
	D	100	100	100	100	100	100	100
	Mean	**100**	**100**	**98.8**	**98.8**	**97.5**	**98.8**	**96.3**
	SD	0.0	0.0	2.5	2.5	2.9	2.5	4.8
	RSD	0.0	0.0	2.5	2.5	3.0	2.5	5.0

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

Table 166: F_1 generation, adult stage: individual total length [cm]; control and 0.32 µg Dexamethasone/L control and 0.32 µg Dexamethasone/L (nominal), respective 0.33 µg Dexamethasone/L (mean measured)

	Control								Nominal concentration: 0.32 µg Dexamethasone/L Mean measured concentration: 0.33 µg Dexamethasone/L							
	Total length [cm]															
Replicate	A		B		C		D		A		B		C		D	
Fish No.	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	3.9	-	-(*)	-(*)	-	4.0	-	3.9	-	4.0	-	4.1	-	3.6	4.0	
2	3.9	-	-(*)	-(*)	-	3.8	-	3.6	-	4.0	-	3.7	-	3.5	-	3.8
3	-	3.8	-(*)	-(*)	-	3.8	-	3.6	-	4.1	-	4.0	-	3.6	-	3.9
4	4.0	-	-(*)	-(*)	3.8	-	-	3.5	-	4.1	-	3.9	-	3.6	-	3.5
5	-	4.0	-(*)	-(*)	-	4.0	3.6	-	-	3.8	-	4.0	4.0		-	3.7
6	-	3.5	-(*)	-(*)	-	3.9	3.6	-	3.8	-	-	4.0	3.6		-	3.8
7	3.7	-	-(*)	-(*)	-	3.7	3.6	-	-	3.5	3.9	-	3.5		-	3.6
8	4.0	-	-(*)	-(*)	3.6	-	-	3.9	-	3.7	-	3.9	3.7		-	3.6
9	3.7	-	-(*)	-(*)	3.6	-	-	4.0	3.8	-	4.0	-	-	3.9	-	3.6
10	-	3.8	-(*)	-(*)	4.0	-	-	4.0	3.9	-	3.9	-	-	3.7	3.9	-
11	-	3.6	-(*)	-(*)	-	3.5	-	3.6	3.6	-	4.0	-	-	3.6	-	3.9
12	-	3.6	-(*)	-(*)	3.7	-	-	3.6	4.0	-	-	4.0	-	4.0	-	3.6
13	-	3.5	-(*)	-(*)	4.0	-	3.8	-	-	3.7	-	3.9	-	3.7	-	3.9
14	-	3.5	-(*)	-(*)	3.6	-	3.6	-	3.9	-	4.0	-	3.9	-	-	4.0
15	4.0	-	-(*)	-(*)	3.5	-	-	3.7	-	4.1	-	4.0	3.7	-	-	3.6

	Control								**Nominal concentration: 0.32 µg Dexamethasone/L Mean measured concentration: 0.33 µg Dexamethasone/L**							
16	3.9	-	-(*)	-(*)	-	3.6	-	4.0	-	4.0	-	4.1	-	4.0	-	3.7
17	-	3.6	-(*)	-(*)	-	4.0	-	3.7	-	3.9	-	4.1	-	3.5	3.7	-
18	-	3.5	-(*)	-(*)	-	3.9	3.8	-	4.0	-	-	3.9	3.8		-	3.5
19	3.9	-	-(*)	-(*)	-	3.8	3.5	-	4.0	-	3.9	-	-	3.8	3.8	-
20	3.8	-	-(*)	-(*)	3.9	-	-	3.6	4.0	-	4.1	-	-	3.5	3.9	-
Mean	**3.9**	**3.6**	**-(*)**	**-(*)**	**3.7**	**3.8**	**3.6**	**3.7**	**3.9**	**3.9**	**4.0**	**4.0**	**3.7**	**3.7**	**3.9**	**3.7**
SD	0.1	0.2	-	-	0.2	0.2	0.1	0.2	0.1	0.2	0.1	0.1	0.2	0.2	0.1	0.2
RSD	2.9	4.7	-	-	5.0	4.4	3.1	4.9	3.5	5.1	1.9	2.8	4.6	4.9	3.0	4.3

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

Table 167: F_1 generation, adult stage: individual total length [cm]; 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.91 and 3.19 µg Dexamethasone/L (mean measured)

	Nominal concentration: 1.00 µg Dexamethasone/L Mean measured concentration: 0.91 µg Dexamethasone/L								Nominal concentration: 3.20 µg Dexamethasone/L Mean measured concentration: 3.19 µg Dexamethasone/L							
	Total length [cm]															
Replicate	A		B		C		D		A		B		C		D	
Fish No.	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	-	4.0	-	4.1	-	3.8	3.8		-	3.5	-	4.0	3.9	-	-	4.0
2	-	4.0	-	4.3	-	4.1	-	3.5	-	3.8	4.0	-	3.8	-	-	4.0
3	-	4.0	-	4.0	3.8	-	-	3.9	-	3.9	-	4.0	-	3.8	-	4.0
4	-	3.7	3.9	-	-	3.8	-	3.7	-	4.1	3.8	-	-	3.5	3.8	-
5	-	4.0	-	4.0	3.8	-	-	3.8	-	3.9	-	3.8	3.6	-	-	3.8
6	-	3.8	-	4.0	4.0	-	-	3.9	-	3.6	-	4.0	4.0	-	-	4.0
7	-	4.0	-	3.9	3.5	-	-	3.6	-	3.9	-	4.2	4.0	-	-	4.0
8	-	3.8	-	3.6	3.5	-	-	3.6	-	3.6	-	3.9	3.8	-	3.7	-
9	-	4.0	-	4.0	-	3.6	3.6	-	-	3.5	-	3.6	4.1	-	-	3.9
10	-	4.0	-	4.1	-	3.7	4.0	-	-	3.5	3.8	-	4.0	-	-	4.2
11	-	3.9	-	4.0	-	3.6	3.8	-	3.8	-	3.9	-	-	3.3	-	3.8
12	3.8	-	3.1	-	-	3.9	-	3.3	-	3.5	3.8	-	-	3.2	-	3.8
13	3.6	-	3.5	-	-	3.8	-	3.4	3.8	-	3.9	-	-	4.0	-	4.0
14	-	4.0	4.0	-	3.5	-	-	4.0	4.0	-	4.0	-	-	3.3	-	4.0
15	-	3.8	-	4.0	3.8	-	-	3.7	-	-	-	3.7	3.7	-	3.9	-

	Nominal concentration: 1.00 µg Dexamethasone/L Mean measured concentration: 0.91 µg Dexamethasone/L								**Nominal concentration: 3.20 µg Dexamethasone/L Mean measured concentration: 3.19 µg Dexamethasone/L**							
16	-	4.2	-	4.0	3.7	-	-	4.2	3.9	3.9	-	3.9	3.8	-	3.8	-
17	-	3.8	-	4.1	-	3.5	-	4.0	-	3.8	-	4.0	3.9	-	-	4.0
18	-	3.6	-	3.6	3.9		3.9	-	-	3.9	4.0	-	3.6	-	-	4.0
19	4.0	-	-	3.7	-	3.6	3.9	-	-	3.7	-	4.0	4.0	-	-	3.9
20	-	3.8	-	3.6	-	-	4.0	-	-		4.1	-	-	-	-	3.8
Mean	**3.8**	**3.9**	**3.6**	**3.9**	**3.7**	**3.7**	**3.9**	**3.7**	**3.9**	**3.7**	**3.9**	**3.9**	**3.9**	**3.5**	**3.8**	**4.0**
SD	0.2	0.1	0.4	0.2	0.2	0.2	0.1	0.3	0.1	0.2	0.1	0.2	0.2	0.3	0.1	0.1
RSD	5.3	3.8	11.3	5.2	5.0	4.7	3.6	7.0	2.5	5.2	2.8	4.2	4.2	9.1	2.1	2.8

Table 168: F_1 generation, adult stage: individual total length [cm]; 10.0 and 32.0 µg Dexamethasone/L (nominal), respective 10.5 and 34.7 µg Dexamethasone (mean measured)

	Nominal concentration: 10.0 µg Dexamethasone/L Mean measured concentration: 10.5 µg Dexamethasone/L								Nominal concentration: 32.0 µg Dexamethasone/L Mean measured concentration: 34.7 µg Dexamethasone/L							
	Total length [cm]															
Replicate	A		B		C		D		A		B		C		D	
Fish No.	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	-	4.0	-	3.8	3.6	-	-	4.0	-	3.9	-	4.0	-	3.6	-	3.5
2	-	3.9	4.0(*)	-	-	3.7	-	3.6	-	3.8	-	4.0	3.7	-	-	3.5
3	-	3.8	-	3.7	-	3.7	-	4.0	3.7	-	-	3.9	-	3.8	-	3.5
4	-	3.7	-	3.8	-	4.0	-	3.9	-	3.5	-	3.5	-	3.6	-	3.5
5	-	3.6	-	3.8	-	4.0	3.6	-	-	3.8	-	4.0	-	3.3	3.5	-
6	-	4.3	-	3.6	4.0	-	3.6	-	-	3.6	-	4.0	-	3.5	3.6	-
7	3.9	-	3.5	-	4.0	-	3.8	-	-	3.8	-	4.1	3.5	-	-	3.7
8	4	-	3.6	-	3.8	-	3.7	-	-	3.9	-	3.9	3.2	-	-	3.5
9	4.0	-	3.7	-	-	3.9	-	3.9	-	3.7	-	3.6	3.5	-	3.6	-
10	-	3.8	3.8	-	4.0	-	-	3.8	-	4.0	-	3.7	3.5(*)	-	-	3.6
11	-	3.8	4.0	-	-	4.5	-	4.0	-	3.9	3.6	-	3.5	-	3.2	-
12	3.9	-	3.6	-	-	3.8	-	3.9	-	3.8	3.6	-	-	3.6	-	3.3
13	4	-	-	3.9	-	3.8	-	4.0	-	3.9	3.8	-	-	3.5	-	3.5
14	4.0	-	-	4.0	4.0	-	4.0	-	-	3.8	3.6	-	-	3.8	-	3.6
15	-	3.5	3.8	-	-	4.0	3.5	-	-	3.9	-	3.5	-	3.3	-	3.5

	Nominal concentration: 10.0 µg Dexamethasone/L Mean measured concentration: 10.5 µg Dexamethasone/L								Nominal concentration: 32.0 µg Dexamethasone/L Mean measured concentration: 34.7 µg Dexamethasone/L							
16	3.8	-	3.8	-	3.8	-	-	3.9	-	3.8	3.7	-	3.7	-	-	3.5
17	3.5	-	-	3.8	3.8	-	3.7	-	-	3.5	-	3.8	3.5	-	-	3.9
18	3.8	-	3.8	-	-	3.5	-	3.5	3.5	-	-	3.9	3.3	-	-	3.6
19	3.9	-	3.8	-	3.6	-	3.8	-	-	3.6	3.8	-	3.5	-	-	3.6
20	-	-	3.8	-	-	-	-	4.0	3.8	-	-	-	-	3.3	-	3.4
Mean	**3.9**	**3.8**	**3.7**	**3.8**	**3.8**	**3.9**	**3.7**	**3.9**	**3.7**	**3.8**	**3.7**	**3.8**	**3.5**	**3.5**	**3.5**	**3.5**
SD	0.2	0.2	0.1	0.1	0.2	0.3	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.1
RSD	4.0	6.1	3.1	3.1	4.3	6.9	4.2	4.3	4.2	3.9	2.7	5.3	4.6	5.4	5.4	3.7

(*) Both fishes were determined as hermaphrodite and thus not included in the calculation of the mean.

Table 169: F_1 generation, adult stage: individual total length [cm]; 100 µg Dexamethasone/L (nominal), respective 100 µg Dexamethasone/L (mean measured)

	Nominal concentration: 100 µg Dexamethasone/L Mean measured concentration: 100 µg Dexamethasone/L							
	Total length [cm]							
Replicate	**A**		**B**		**C**		**D**	
Fish No.	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
1	-	3.5	-	3.8	-	3.8	3.5	-
2	3.2	-	-	3.9	3.5	-	-	3.6
3	3.3	-	-	3.9	3.4	-	3.5	-
4	3.4	-	-	3.9	-	3.8	3.2	-
5	3.6	-	-	3.6	-	3.1	-	3.5
6	3.6	-	-	3.6	-	3.3	-	3.0
7	-	3.8	-	3.5	-	3.7	-	3.7
8	-	3.5	-	3.7	3.5	-	-	3.6
9	-	3.2	-	3.6	3.3	-	-	3.3
10	3.1	-	-	3.6	-	3.5	-	3.3
11	3.5	-	-	3.8	3.5	-	-	3.2
12	3.5	-	-	3.8	-	3.5	-	3.5
13	3.5	-	-	3.5	-	3.8	-	3.3
14	-	3.7	-	3.7	-	3.5	3.5	-
15	-	3.5	-	3.5	3.6	-	-	3.5

	Nominal concentration: 100 µg Dexamethasone/L **Mean measured concentration: 100 µg Dexamethasone/L**							
16	-	3.7	-	3.6	-	3.7	-	3.5
17	3.5	-	3.6	-	-	3.8	-	3.6
18	-	3.4	3.5	-	-	3.7	3.5	-
19	-	-	-	3.6	-	3.5	-	3.5
20	-	-	-	-	-	3.5	-	3.4
Mean	**3.4**	**3.5**	**3.6**	**3.7**	**3.5**	**3.6**	**3.4**	**3.4**
SD	0.2	0.2	0.1	0.1	0.1	0.2	0.1	0.2
RSD	4.9	5.4	2.0	3.9	3.0	5.9	3.9	5.4

Table 170: F_1 generation, adult stage: individual wet weight [g]; control and 0.32 µg Dexamethasone/L (nominal), respective 0.33 µg Dexamethasone/L (mean measured)

	Control								Nominal concentration: 0.32 µg Dexamethasone/L Mean measured concentration: 0.33 µg Dexamethasone/L							
	Wet weight [g]															
Replicate	A		B		C		D		A		B		C		D	
Fish No.	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	0.431		-(*)	-(*)	-	0.518	-	0.529	-	0.718	-	0.625	-	0.554	0.472	-
2	0.435		-(*)	-(*)	-	0.545	-	0.574	-	0.714	-	0.503	-	0.502	-	0.596
3		0.557	-(*)	-(*)	-	0.545	-	0.518	-	0.690	-	0.600	-	0.476	-	0.566
4	0.511		-(*)	-(*)	0.440	-	-	0.368	-	0.764	-	0.534	-	0.475	-	0.424
5		0.600	-(*)	-(*)	-	0.629	0.413	-	-	0.575	-	0.667	0.469	-	-	0.513
6		0.462	-(*)	-(*)	-	0.601	0.429	-	0.413	-	-	0.552	0.400	-	-	0.511
7	0.402		-(*)	-(*)	-	0.487	0.409	-	-	0.438	0.448	-	0.380	-	-	0.451
8	0.515		-(*)	-(*)	0.374	-	-	0.560	-	0.534	-	0.537	0.388	-	-	0.438
9	0.451		-(*)	-(*)	0.366	-	-	0.578	0.463	-	0.537	-	-	0.602	-	0.434
10		0.542	-(*)	-(*)	0.476	-	-	0.608	0.459	-	0.441	-	-	0.529	0.475	-
11		0.478	-(*)	-(*)	-	0.460	-	0.472	0.350	-	0.541	-	-	0.527	-	0.695
12		0.561	-(*)	-(*)	0.395	-	-	0.470	0.476	-	-	0.702	-	0.592	-	0.526
13		0.435	-(*)	-(*)	0.556	-	0.444	-	-	0.454	-	0.609	-	0.545	-	0.588
14		0.471	-(*)	-(*)	0.346	-	0.389	-	0.389	-	0.542	-	0.427	-	-	0.611
15	0.468		-(*)	-(*)	0.319	-	-	0.499	-	0.836	-	0.609	0.451	-	-	0.519

	Control								Nominal concentration: 0.32 µg Dexamethasone/L Mean measured concentration: 0.33 µg Dexamethasone/L							
16	0.417		-(*)	-(*)	-	0.571	-	0.605	-	0.590	-	0.742	-	0.560	-	0.546
17		0.542	-(*)	-(*)	-	0.679	-	0.480	-	0.467	-	0.741	-	0.443	0.432	-
18		0.438	-(*)	-(*)	-	0.569	0.461	-	0.520	-	-	0.589	0.432	-	-	0.510
19	0.424		-(*)	-(*)	-	0.540	0.352	-	0.429	-	0.487	-	-	0.570	0.410	-
20	0.390		-(*)	-(*)	0.475	-	-	0.466	0.518	-	0.583	-	-	0.524	0.400	-
Mean	**0.444**	**0.509**	**-(*)**	**-(*)**	**0.416**	**0.559**	**0.414**	**0.517**	**0.446**	**0.616**	**0.511**	**0.616**	**0.421**	**0.531**	**0.438**	**0.529**
SD	0.042	0.058	-	-	0.076	0.062	0.036	0.068	0.057	0.136	0.053	0.078	0.033	0.047	0.035	0.075
RSD	9.6	11.5	-	-	18.3	11.1	8.7	13.2	12.7	22.1	10.5	12.6	7.9	8.9	7.9	14.2

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

Table 171: F_1 generation, adult stage: individual wet weight [g]; 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.91 and 3.19 µg Dexamethasone/L (mean measured)

	Nominal concentration: 1.00 µg Dexamethasone/L Mean measured concentration: 0.91 µg Dexamethasone/L								Nominal concentration: 3.20 µg Dexamethasone/L Mean measured concentration: 3.19 µg Dexamethasone/L							
	Wet weight [g]															
Replicate	A		B		C		D		A		B		C		D	
Fish No.	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	-	0.607	-	0.719	-	0.624	0.449	-	-	0.531	-	0.653	0.444	-	-	0.585
2	-	0.540	-	0.716	-	0.708	-	0.471	-	0.465	0.540	-	0.418	-	-	0.604
3	-	0.583	-	0.570	0.493	-	-	0.650	-	0.697	-	0.592	-	0.538	-	0.562
4	-	0.589	0.448	-	-	0.476	-	0.530	-	0.734	0.415	-	-	0.386	0.409	-
5	-	0.717	-	0.555	0.487	-	-	0.525	-	0.546	-	0.589	0.355	-	-	0.523
6	-	0.591	-	0.601	0.493	-	-	0.631	-	0.411	-	0.658	0.438	-	-	0.513
7	-	0.616	-	0.512	0.423	-	-	0.513	-	0.583	-	0.816	0.507	-	-	0.521
8	-	0.604	-	0.468	0.364	-	-	0.520	-	0.592	-	0.562	0.402	-	0.435	-
9	-	0.588	-	0.745	-	0.432	0.444	-	-	0.437	-	0.479	0.510	-	-	0.513
10	-	0.563	-	0.788	-	0.506	0.510	-	-	0.414	0.458	-	0.452	-	-	0.700
11	-	0.494	-	0.650	-	0.526	0.391	-	0.465	-	0.460	-	-	0.385	-	0.476
12	0.410	-	0.244	-	-	0.590	-	0.400	-	0.479	0.443	-	-	0.411	-	0.471
13	0.373	-	0.422	-	-	0.541	-	0.411	0.423	-	0.470	-	-	0.637	-	0.509
14	-	0.584	0.490	-	0.387	-	-	0.652	0.471	-	0.507	-	-	0.400	-	0.520
15	-	0.497	-	0.603	0.447	-	-	0.473	-	0.520	-	0.514	0.490	-	0.383	-

	Nominal concentration: 1.00 µg Dexamethasone/L Mean measured concentration: 0.91 µg Dexamethasone/L								**Nominal concentration: 3.20 µg Dexamethasone/L Mean measured concentration: 3.19 µg Dexamethasone/L**							
16	-	0.643	-	0.766	0.416	-	-	0.685	0.494	-	-	0.516	0.464	-	0.429	-
17	-	0.491	-	0.600	-	0.464	-	0.632	-	0.559	-	0.599	0.484	-	-	0.626
18	-	0.507	-	0.555	0.478	-	0.440	-	-	0.535	0.526	-	0.383	-	-	0.537
19	0.460	-	-	0.560	-	0.490	0.468	-	-	0.489	-	0.650	0.490	-	-	0.525
20	-	0.506	-	0.464	-	-	0.577	-	-	-	0.565	-	-	-	-	0.497
Mean	**0.414**	**0.572**	**0.401**	**0.617**	**0.443**	**0.536**	**0.468**	**0.546**	**0.463**	**0.533**	**0.487**	**0.603**	**0.449**	**0.460**	**0.414**	**0.543**
SD	0.044	0.061	0.108	0.103	0.048	0.084	0.060	0.095	0.030	0.093	0.050	0.093	0.049	0.104	0.023	0.060
RSD	10.5	10.7	27.0	16.7	10.9	15.6	12.7	17.5	6.4	17.5	10.2	15.4	10.8	22.7	5.7	11.0

Table 172: F_1 generation, adult stage: individual wet weight [g]; 10.0 and 32.0 µg Dexamethasone/L (nominal), respective 10.5 and 34.7 µg Dexamethasone (mean measured)

	Nominal concentration: 10.0 µg Dexamethasone/L Mean measured concentration: 10.5 µg Dexamethasone/L								Nominal concentration: 32.0 µg Dexamethasone/L Mean measured concentration: 34.7 µg Dexamethasone/L							
	Wet weight [g]															
Replicate	A		B		C		D		A		B		C		D	
Fish No.	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
1	-	0.634	-	0.606	0.349	-	-	0.596	-	0.547	-	0.531	-	0.464	-	0.380
2	-	0.556	0.585(*)	-	-	0.597	-	0.498	-	0.464	-	0.543	0.406	-	-	0.406
3	-	0.497	-	0.480	-	0.511	-	0.581	0.362	-	-	0.450	-	0.517	-	0.423
4	-	0.480	-	0.523	-	0.489	-	0.547	-	0.395	-	0.400	-	0.503	-	0.430
5	-	0.468	-	0.555	-	0.525	0.387	-	-	0.436	-	0.520	-	0.346	0.372	-
6	-	0.649	-	0.420	0.459	-	0.380	-	-	0.392	-	0.649	-	0.383	0.333	-
7	0.434	-	0.342	-	0.426	-	0.383	-	-	0.427	-	0.743	0.355	-	-	0.435
8	0.490	-	0.386	-	0.392	-	0.385	-	-	0.488	-	0.565	0.283	-	-	0.396
9	0.426	-	0.418	-	-	0.450	-	0.534	-	0.440	-	0.475	0.360	-	0.367	-
10	-	0.527	0.474	-	0.440	-	-	0.534	-	0.574	-	0.498	0.428(*)	-	-	0.388
11	-	0.482	0.471	-	-	0.911	-	0.602	-	0.546	0.333	-	0.372	-	0.320	-
12	0.467	-	0.399	-	-	0.482	-	0.516	-	0.456	0.317	-	-	0.446	-	0.368
13	0.479	-	-	0.588	-	0.482	-	0.510	-	0.514	0.394	-	-	0.492	-	0.403
14	0.447	-	-	0.540	0.446	-	0.411	-	-	0.476	0.386	-	-	0.457	-	0.399
15	-	0.436	0.385	-	-	0.528	0.367	-	-	0.512	-	0.453	-	0.384	-	0.383

	Nominal concentration: 10.0 µg Dexamethasone/L Mean measured concentration: 10.5 µg Dexamethasone/L								**Nominal concentration: 32.0 µg Dexamethasone/L Mean measured concentration: 34.7 µg Dexamethasone/L**							
16	0.454	-	0.425	-	0.405	-	-	0.564	-	0.469	0.361	-	0.410	-	-	0.404
17	0.435	-	-	0.510	0.422	-	0.400	-	-	0.388	-	0.437	0.383	-	-	0.522
18	0.333	-	0.440	-	-	0.342	-	0.357	0.320	-	-	0.520	0.310	-	-	0.422
19	0.454	-	0.406	-	0.367	-	0.433	-	-	0.455	0.406	-	0.417	-	-	0.394
20	-	-	0.409	-	-	-	-	0.608	0.386	-	-	-	-	0.353	-	0.391
Mean	**0.442**	**0.525**	**0.414**	**0.528**	**0.412**	**0.532**	**0.393**	**0.537**	**0.356**	**0.469**	**0.366**	**0.522**	**0.366**	**0.435**	**0.348**	**0.409**
SD	0.043	0.074	0.038	0.060	0.037	0.148	0.021	0.068	0.033	0.056	0.035	0.092	0.046	0.063	0.025	0.035
RSD	9.8	14.1	9.3	11.3	9.0	27.9	5.3	12.6	9.4	11.8	9.7	17.7	12.5	14.6	7.3	8.7

(*) Both fishes were determined as hermaphrodite and thus excluded from the calculation of the mean.

Table 173: F_1 generation, adult stage: individual wet weight [g]; 100 µg Dexamethasone/L (nominal), respective 100 µg Dexamethasone/L (mean measured)

	Nominal concentration: 100 µg Dexamethasone/L Mean measured concentration: 100 µg Dexamethasone/L							
	Wet weight [g]							
Replicate	A		B		C		D	
Fish No.	Male	Female	Male	Female	Male	Female	Male	Female
1	-	0.425	-	0.479	-	0.449	0.313	-
2	0.283	-	-	0.553	0.302	-	-	0.377
3	0.264	-	-	0.581	0.269	-	0.309	-
4	0.275	-	-	0.542	-	0.462	0.256	-
5	0.295	-	-	0.443	-	0.252	-	0.381
6	0.331	-	-	0.404	-	0.334	-	0.242
7	-	0.421	-	0.313	-	0.410	-	0.384
8	-	0.378	-	0.421	0.315	-	-	0.390
9	-	0.269	-	0.410	0.256	-	-	0.320
10	0.252	-	-	0.409	-	0.368	-	0.300
11	0.312	-	-	0.554	0.319	-	-	0.249
12	0.301	-	-	0.454	-	0.352	-	0.368
13	0.318	-	-	0.364	-	0.444	-	0.306
14	-	0.461	-	0.458	-	0.329	0.293	-
15	-	0.413	-	0.414	0.325	-	-	0.353

	Nominal concentration: 100 µg Dexamethasone/L **Mean measured concentration: 100 µg Dexamethasone/L**							
16	-	0.479	-	0.406	-	0.392	-	0.370
17	0.334	-	0.323	-	-	0.448	-	0.403
18	-	0.340	0.270	-	-	0.448	0.293	-
19	-	-	-	0.355	-	0.394	-	0.392
20	-	-	-	-	-	0.350	-	0.319
Mean	**0.297**	**0.398**	**0.297**	**0.445**	**0.298**	**0.388**	**0.293**	**0.344**
SD	0.028	0.068	0.037	0.076	0.029	0.061	0.022	0.052
RSD	9.4	17.1	12.6	17.1	9.6	15.7	7.7	15.0

Table 174: F_1 generation, adult stage, summary: mean total length and mean wet weight

		Nominal concentration Dexamethasone [µg/L]						
		control	0.32	1.00	3.20	10.0	32.0	100
		Mean measured concentration Dexamethasone [µg/L]						
	Replicate	Control	0.33	0.91	3.19	10.5	34.7	100
Total length males, adult stage [cm]	A	3.9	3.9	3.8	3.9	3.9	3.7	3.4
	B	-(*)	4.0	3.6	3.9	3.7	3.7	3.6
	C	3.7	3.7	3.7	3.9	3.9	3.5	3.5
	D	3.6	3.9	3.9	3.8	3.7	3.5	3.4
	Mean	**3.8**	**3.9**	**3.8**	**3.9**	**3.8**	**3.6(**)**	**3.5(**)**
	SD	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	RSD	3.2	2.4	2.7	1.3	2.4	3.1	1.6
Total length females, adult stage [cm]	A	3.6	3.9	3.9	3.7	3.8	3.8	3.5
	B	-(*)	4.0	3.9	3.9	3.8	3.8	3.7
	C	3.8	3.7	3.7	3.5	3.9	3.5	3.6
	D	3.7	3.7	3.7	4.0	3.9	3.5	3.4
	Mean	**3.7**	**3.8**	**3.8**	**3.8**	**3.8**	**3.7**	**3.6**
	SD	0.1	0.1	0.1	0.2	0.0	0.2	0.1
	RSD	2.4	3.6	2.8	5.3	1.1	4.3	2.9
Wet weight males, adult stage [g]	A	0.444	0.446	0.414	0.463	0.442	0.356	0.297
	B	-(*)	0.511	0.401	0.487	0.414	0.366	0.297
	C	0.416	0.421	0.443	0.449	0.532	0.366	0.298
	D	0.414	0.438	0.468	0.414	0.393	0.348	0.293
	Mean	**0.425**	**0.454**	**0.432**	**0.453**	**0.445**	**0.359(**)**	**0.296(**)**
	SD	0.017	0.040	0.030	0.031	0.061	0.009	0.002
	RSD	4.0	8.7	7.0	6.7	13.7	2.5	0.7

		Nominal concentration Dexamethasone [µg/L]						
Wet weight females adult stage [g]	A	0.509	0.616	0.572	0.533	0.525	0.469	0.398
	B	-(*)	0.616	0.617	0.603	0.528	0.522	0.445
	C	0.559	0.531	0.536	0.460	0.532	0.435	0.388
	D	0.517	0.529	0.546	0.543	0.537	0.409	0.344
	Mean	**0.528**	**0.573**	**0.568**	**0.534**	**0.531**	**0.459(**)**	**0.394(**)**
	SD	0.027	0.050	0.036	0.059	0.005	0.049	0.041
	RSD	5.0	8.7	6.4	11.0	1.0	10.6	10.5

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

(**) Statistically significant reduction compared to control, $p<0.05$, Williams test, one-sided smaller.

Table 175: F_1 generation, sex ratio; control, 0.32, 1.00 and 3.20 µg Dexamethasone/L (nominal) respective 0.33, 0.91 and 3.19 µg Dexamethasone/L (mean measured) (based on histological data provided in C.2.14)

	Nominal concentration Dexamethasone [µg/L]															
	Control				**0.32**				**1.00**				**3.20**			
	Mean measured concentration Dexamethasone [µg/L]															
	Control				**0.33**				**0.91**				**3.19**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Male [n]	10	-(*)	9	7	9	7	7	5	3	4	9	7	4	9	13	4
Female [n]	10	-(*)	11	13	11	13	13	15	17	16	10	13	15	11	6	16
Hermaphrodite [n]	0	-(*)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total [n]	20	-(*)	20	20	20	20	20	20	20	20	19	20	19	20	19	20
Male [%]	50.0	-(*)	45.0	35.0	45.0	35.0	35.0	25.0	15.0	20.0	47.4	35.0	21.1	45.0	68.4	20.0
Female [%]	50.0	-(*)	55.0	65.0	55.0	65.0	65.0	75.0	85.0	80.0	52.6	65.0	78.9	55.0	31.6	80.0
Hermaphrodite [%]	0.0	-(*)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total [%]	100	-(*)	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Total mean male [%]				**43.3**				**35.0**				**29.3**				**38.6**
SD				7.6				8.2				14.7				23.0
RSD				17.6				23.3				50.2				59.5
Total mean female [%]				**56.7**				**65.0**				**70.7**				**61.4**
SD				7.6				8.2				14.7				23.0
RSD				13.5				12.6				20.8				37.4

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

Table 176: F_1 generation, sex ratio, 10.0. 32.0 and 100 µg Dexamethasone/L (based on histological data provided in C.2.14)

	Nominal concentration Dexamethasone [µg/L]											
	10.0				**32.0**				**100**			
	Mean measured concentration Dexamethasone [µg/L]											
	10.5				**34.7**				**100**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Male [n]	10	11	9	8	3	6	9	4	10	2	6	5
Female [n]	9	8	10	12	17	13	10	16	8	17	14	15
Hermaphrodite [n]	0	1	0	0	0	0	1	0	0	0	0	0
Total [n]	19	20	19	20	20	19	20	20	18	19	20	20
Male [%]	52.6	55.0	47.4	40.0	15.0	31.6	45.0	20.0	55.6	10.5	30.0	25.0
Female [%]	47.4	40.0	52.6	60.0	85.0	68.4	50.0	80.0	44.4	89.5	70.0	75.0
Hermaphrodite [%]	0.0	5.0	0.0	0.0	0.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0
Total [%]	100	100	100	100	100	100	100	100	100	100	100	100
Total mean male [%]	**48.8**				**27.9**				**30.3**			
SD	6.6				13.4				18.8			
RSD	13.6				47.9				62.0			
Total mean female [%]	**50.0**				**70.9**				**69.7**			
SD	8.4				15.5				18.8			
RSD	16.9				21.9				26.9			
Total mean hermaphrodite [%]	**1.3**				**1.3**				**0.0**			
SD	2.5				2.5				-			
RSD	200				200				-			

C.2.10 Second filial generation (F_2)

Table 177: Second filial generation (F_2), hatching success

		Nominal concentration Dexamethasone [µg/L]						
		Control	0.32	1.00	3.20	10.0	32.0	100
		Mean measured concentration Dexamethasone [µg/L]						
	Replicate	Control	0.33	0.91	3.19	10.5	34.7	100
Introduced eggs [n]	A	20	20	20	20	20	20	20
	B	-(*)	20	20	20	20	20	20
	C	20	20	20	20	20	20	20
	D	20	20	20	20	20	20	20
Coagulated eggs [n]	A	0	0	0	1	1	3	1
	B	-(*)	0	0	0	4	1	0
	C	0	0	0	1	1	3	1
	D	0	0	0	0	2	1	0
Hatch, day 4 pf [n]	A	20	20	20	19	19	17	19
	B	-(*)	20	20	20	16	19	20
	C	20	20	20	19	19	17	19
	D	20	20	20	20	18	19	20
Hatch, day 4 pf [%]	A	100	100	100	95.0	95.0	85.0	95.0
	B	-(*)	100	100	100	80.0	95.0	100
	C	100	100	100	95.0	95.0	85.0	95.0
	D	100	100	100	100	90.0	95.0	100
	Mean	**100**	**100**	**100**	**97.5**	**90.0(**)**	**90.0(**)**	**97.5**
	SD	**0.0**	**0.0**	**0.0**	**2.9**	**7.1**	**5.8**	**2.9**
	RSD	**0.0**	**0.0**	**0.0**	**3.0**	**7.9**	**6.4**	**3.0**

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

(**) Statistically significant reduction compared to control, Multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm, one-sided-smaller, heterogenous variances.

C.2.11 Biomarker evaluation, F_0 generation

Table 178: F_0 generation, Vitellogenin content [ng/mL]; control

VTG [ng/mL]	Control							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1	-	1.61E+08	-	1.02E+08	42.84	-	74.32	-
2	781.6	-	109.1	-	16.54	-	-	2.50E+07
3	-	4.69E+07	-	4.90E+07	-	4.57E+07	54.44	-
4	-	4.45E+07	58.33	-	17.96	-	-	2.54E+07
5	10.75	-	-	9.78E+07	1664	-	103.1	-
6	-	7.62E+07	94.64	-	-	5.04E+06	54.44	-
7	80.65	-	-	6.42E+07	33.55		-	2.84E+07
8	152.3	-	92.9	-	-	2.58E+07	51.27	-
9	-	1.09E+08	-	4.50E+07	-	4.96E+07	-	3.08E+07
10	*<40*	-	47.43	-	-	2.10E+07	-	2.88E+07
Geomean	**100.79**	**7.75E+07**	**76.70**	**6.76E+07**	**58.93**	**2.28E+07**	**6.50E+01**	**2.76E+07**

The value highlighted in *italics* was not included in the calculation of the mean.

Table 179: F_0 generation, Vitellogenin content [ng/mL]; 0.32 µg Dexamethasone/L (nominal), respective 0.33 µg Dexamethasone/L (mean measured)

VTG [ng/mL]	0.32 µg Dexamethasone/L (nominal); 0.33 µg Dexamethasone/L (mean measured)							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1	-	9.71E+07	53.79	-	66.96	-	53.16	-
2	-	6.64E+07	-	2.19E+08	-	7.18E+07	67.83	-
3	-	2.39E+07	463.9	-	365.9	-	-	9.00E+07
4	-	4.64E+07	-	1.62E+07	-	1.73E+07	37.44	-
5	-	3.27E+07	-	1.53E+08	808.5	-	-	1.03E+08
6	107.60	-	149.8	-	-	4.57E+07	30.82	-
7	582.50	-	-	1.53E+07	1881	-	-	1.66E+07
8	945.40	-	62.93	-	-	2.46E+07	1119	-
9	19.21	-	-	1.28E+07	261	-	-	2.62E+07
10	312.0	-	204.9	-	-	8.77E+06	-	8.97E+06
Geomean	**204.21**	**4.72E+07**	**136.96**	**4.03E+07**	**395.89**	**2.62E+07**	**85.82**	**3.25E+07**

Table 180: F_0 generation, Vitellogenin content [ng/mL]; 1.00 µg Dexamethasone/L (nominal), respective 0.91 µg Dexamethasone/L (mean measured)

VTG [ng/mL]	1.00 µg Dexamethasone/L (nominal), 0.91 µg Dexamethasone/L (mean measured)							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1	901.7	-	124.7	-	77.96	-	-	1.22E+08
2	-	1.26E+07	-	1.53E+08	-	5.58E+07	-	1.14E+08
3	-	4.04E+07	470.0	-	76.53	-	-	1.83E+07
4	3499	-	-	3.42E+07	-	1.39E+08	-	2.66E+07
5	-	3.30E+07	-	1.56E+07	32.27	-	-	2.37E+07
6	719.1	-	126.9	-	-	6.01E+07	2191	-
7	-	3.42E+07	-	1.64E+07	78.30	-	479.6	-
8	-	3.37E+07	839.3	-	107.6	-	889.7	-
9	1082	-	-	2.49E+07	-	2.82E+07	400.6	-
10	130	-	119.9	-	-	5.23E+07	66.1	-
Geomean	**795.78**	**2.87E+07**	**237.05**	**3.20E+07**	**69.50**	**5.85E+07**	**477.24**	**4.38E+07**

Table 181: F_0 generation, Vitellogenin content [ng/mL]; 3.20 µg Dexamethasone/L (nominal), respective 3.19 µg Dexamethasone/L (mean measured)

VTG [ng/mL]	3.20 µg Dexamethasone/L (nominal), 3.19 µg Dexamethasone/L (mean measured)							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1	115.4	-	183.8	-	*<20*	-	33.79	-
2	24.98	-	-	1.08E+08	-	1.56E+08	-	1.92E+08
3	-	6.74E+07	-	1.51E+08	-	6.64E+07	-	1.69E+08
4	114.9	-	105.1	-	50.69		6.419	-
5	12.28	-	-	3.45E+07	-	4.55E+07	88.29	-
6	-	1.44E+07	63.97	-	16.02	-	-	4.99E+07
7	31.99	-	-	1.86E+07	30.54	-	-	1.40E+07
8	-	2.20E+07	38.59	-	57.70	-	-	2.88E+07
9	-	4.76E+07	-	1.91E+07	-	4.73E+07	20154	-
10	-	1.74E+07	-	1.25E+07	-	2.95E+07	4491	-
Geomean	**41.96**	**2.82E+07**	**83.10**	**3.68E+07**	**34.59**	**5.80E+07**	**280.40**	**5.79E+07**

The value highlighted in *italics* was not included in the calculation of the mean.

Table 182: F_0 generation, Vitellogenin content [ng/mL]; 10.0 µg Dexamethasone/L (nominal), respective 10.5 µg Dexamethasone/L (mean measured)

VTG [ng/mL]	10.0 µg Dexamethasone/L (nominal), 10.5 µg Dexamethasone/L (mean measured)							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1	67.16	-	-	2.95E+08	67.99	-	-	3.02E+07
2	-	1.09E+08	45.23	-	-	4.51E+07	86.22	-
3	90.48	-	-	1.10E+08	-	7.96E+07	-	6.51E+07
4	102.9	-	257.5	-	33.55	-	66.69	-
5	68.64	-	-	4.21E+07	-	1.31E+08	-	2.94E+07
6	-	2.55E+07	-	4.41E+07	30.46	-	53.80	-
7	-	2.24E+07	217.6		473.1	-	-	3.93E+07
8	-	5.30E+07		3.04E+07	1135	-	50.66	
9	-	4.96E+07	119.6	-	-	3.46E+07	-	3.55E+07
10	37.53	-	9864	-	-	2.86E+07	81.86	-
Geomean	**69.41**	**4.39E+07**	**312.70**	**7.12E+07**	**130.13**	**5.41E+07**	**66.32**	**3.81E+07**

Table 183: F_0 generation, Vitellogenin content [ng/mL]; 32.0 µg Dexamethasone/L (nominal), respective 34.7 µg Dexamethasone/L (mean measured)

VTG [ng/mL]	32.0 µg Dexamethasone/L (nominal), 34.7 µg Dexamethasone/L (mean measured)							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1	14.85	-	27.85	-	-	8.11E+07	193.4	-
2	52.09	-	4.70E+05	-	1732	-	-	7.11E+07
3	75.79	-	1.51E+05	-	-	8.13E+07	-	1.63E+08
4	84.98	-	-	7.27E+07	916.4	-	41.41	-
5	-	2.44E+07	-	3.80E+07	-	1.34E+08	76.40	-
6	-	2.96E+07	5728	-	2807	-	-	*>2,0E+08*
7	100.3	-	-	2.85E+07	-	2.09E+07	26.92	
8	-	5.03E+07	4839	-	807.3	-	-	1.81E+08
9	-	5.47E+07	-	4.11E+07	-	5.48E+07	-	5.79E+07
10	-	-	-	6.06E+07	2167	-	496.0	-
Geomean	**54.92**	**3.75E+07**	**8866.08**	**4.56E+07**	**1507.84**	**6.32E+07**	**96.04**	**1.05E+08**

The value highlighted in *italics* was not included in the calculation of the mean.

Table 184: F_0 generation, Vitellogenin content [ng/mL], 100 µg Dexamethasone/L (nominal), respective 100 µg Dexamethasone/L (mean measured)

VTG [ng/mL]	100 µg Dexamethasone/L (nominal), 100 µg Dexamethasone/L (mean measured)							
Replicate	**A**		**B**		**C**		**D**	
Fish No	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**
1	215.1	-	309.4	-	-	9.69E+07	1533	-
2	-	2.16E+07	525.9	-	-	4.41E+07	-	8.99E+07
3	-	3.69E+07	158.0	-	-	4.22E+07	-	4.94E+07
4	126.8	-	237.3	-	-	3.91E+07	-	6.36E+07
5	-	2.23E+07	-	8.50E+07	-	7.72E+07	994.8	-
6	356.4	-	-	5.08E+07	1812	-		3.17E+07
7	-	2.70E+07	-	5.69E+07	221.7	-	1377	-
8	540.0	-	-	1.67E+08	1720	-	-	6.31E+07
9	-	4.38E+07	-	4.97E+07	1896	-	28.33	-
10	3506	-	-	-	250.3	-	86.65	-
Geomean	**449.76**	**2.91E+07**	**279.48**	**7.28E+07**	**800.11**	**5.59E+07**	**348.69**	**5.63E+07**

Table 185: F_0 generation, Vitellogenin content [ng/mL], summary

		Nominal concentration Dexamethasone [µg/L]						
		Control	0.32	1.00	3.2.	10.0	32.0	100
		Mean measured concentration Dexamethasone [µg/L]						
	Replicate	Control	0.33	0.91	3.19	10.5	34.7	100
VTG males [ng/mL]	A	100.79	204.21	795.78	41.96	69.41	54.92	449.76
	B	76.70	136.96	237.05	83.10	312.70	8866.08	279.48
	C	58.93	395.89	69.50	34.59	130.13	1507.84	800.11
	D	65.04	85.82	477.24	280.40	66.32	96.04	348.69
	Mean	**75.36**	**205.72**	**394.89**	**110.01**	**144.64**	**2631.22**	**469.51**
	SD	**18.48**	**135.73**	**315.32**	**115.58**	**115.83**	**4211.09**	**231.23**
	RSD	**24.53**	**65.98**	**79.85**	**105.06**	**80.08**	**160.04**	**49.25**
VTG females [ng/mL]	A	7.75E+07	4.72E+07	2.87E+07	2.82E+07	4.39E+07	3.75E+07	2.91E+07
	B	6.76E+07	4.03E+07	3.20E+07	3.68E+07	7.12E+07	4.56E+07	7.28E+07
	C	2.28E+07	2.62E+07	5.85E+07	5.80E+07	5.41E+07	6.32E+07	5.59E+07
	D	2.76E+07	3.25E+07	4.38E+07	5.79E+07	3.81E+07	1.05E+08	5.63E+07
	Mean	**4.89E+07**	**3.65E+07**	**4.07E+07**	**4.52E+07**	**5.19E+07**	**6.28E+07**	**5.35E+07**
	SD	**2.77E+07**	**9.16E+06**	**1.35E+07**	**1.51E+07**	**1.45E+07**	**3.01E+07**	**1.81E+07**
	RSD	**56.66**	**25.07**	**33.21**	**33.46**	**27.97**	**47.88**	**33.73**

C.2.12 Biomarker evaluation, F_1 generation

Table 186: F_1 generation, Vitellogenin content [ng/mL]; control

VTG [ng/mL]	Control							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1	9.586	-	-(*)	-(*)	-	5.02E+07	-	2.68E+07
2	11.54	-	-(*)	-(*)	-	1.90E+07	-	1.13E+07
3	-	6.26E+06	-(*)	-(*)	-	1.47E+07	-	2.41E+07
4	9.587		-(*)	-(*)	17.55	-	-	9.08E+06
5	-	1.47E+06	-(*)	-(*)	-	4.81E+07	58.61	-
6	-	1.07E+07	-(*)	-(*)	-	4.13E+07	17.95	-
7	127	-	-(*)	-(*)	-	7.13E+07	47.12	-
8	158.1	-	-(*)	-(*)	25.16	-	-	7.26E+07
9	219.5	-	-(*)	-(*)	12.77	-	-	8.58E+07
10	-	1.40E+07	-(*)	-(*)	25.18	-	-	6.77E+07
11	-	3.96E+07	-(*)	-(*)	-	2.45E+07	-	5.01E+07
12	-	2.26E+07	-(*)	-(*)	13.07	-	-	1.14E+08
13	-	1.10E+07	-(*)	-(*)	22.56	-	31.20	-
14	-	3.11E+07	-(*)	-(*)	15.43	-	8.721	-
15	27.87	-	-(*)	-(*)	56.27	-	-	3.23E+07
16	18.85	-	-(*)	-(*)	-	1.17E+08	-	4.22E+07
17	-	2.12E+07	-(*)	-(*)	-	2.02E+08	-	4.84E+07
18	-	8.93E+06	-(*)	-(*)	-	4.72E+07	25.39	-
19	41.32	-	-(*)	-(*)	-	4.76E+07	31.10	-
20	32.01	-	-(*)	-(*)	26.32	-	-	5.26E+04
Geomean	**35.58**	**1.23E+07**	**-(*)**	**-(*)**	**21.44**	**4.72E+07**	**27.07**	**2.32E+07**

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

Table 187: F_1 generation, Vitellogenin content [ng/mL]; 0.32 µg Dexamethasone/L (nominal), respective 0.33 µg Dexamethasone/L (mean measured)

VTG [ng/mL]	0.32 µg Dexamethasone/L (nominal); 0.33 µg Dexamethasone/L (mean measured)							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1	-	1.03E+07	-	1.02E+07	-	2.53E+07	7.26	-
2	-	1.47E+07	-	1.32E+03	-	1.44E+07	-	2.39E+07
3	-	2.07E+07	-	9.97E+06	-	2.98E+07	-	1.81E+07
4	-	2.80E+07	-	4.06E+06	-	2.64E+07	-	2.52E+07
5	-	1.05E+04	-	3.54E+06	53.06	-	-	8.17E+06
6	40.10	-	-	1.48E+07	20.16	-	-	1.91E+07
7	-	4.47E+06	41.37		179.5	-	-	1.53E+07
8	-	1.41E+07	-	6.50E+06	34.24	-	-	7.76E+06
9	40.50	-	19.71	-	-	3.04E+07	-	1.77E+07
10	13.97	-	40.54	-	-	1.06E+07	*<30*	-
11	30.08	-	19.04	-	-	9.77E+06	-	3.34E+07
12	39.91	-	-	3.42E+06	-	2.16E+07	-	3.67E+07
13	-	1.67E+05	-	1.66E+07	-	1.85E+07	-	3.41E+07
14	15.80	-	16.41	-	361.4	-	-	1.23E+07
15	-	1.25E+05	-	3.10E+07	63.85	-	-	2.50E+07
16	-	3.97E+07	-	1.08E+07	-	2.09E+07	-	3.63E+07
17	-	3.02E+04	-	1.92E+07	-	9.44E+06	62.73	-
18	50.55	-	-	4.78E+06	49.83	-	-	2.24E+07
19	143.7	-	23.91	-	-	7.80E+06	38.10	-
20	21.43	-	313.3	-	-	3.67E+07	27.44	-
Geomean	**34.37**	**1.94E+06**	**35.93**	**4.51E+06**	**69.15**	**1.80E+07**	**26.27**	**2.02E+07**

The value highlighted in *italics* was not included in the calculation of the mean.

Table 188: F_1 generation, Vitellogenin content [ng/mL]; 1.00 µg Dexamethasone/L (nominal), respective 0.91 µg Dexamethasone/L (mean measured)

VTG [ng/mL]	1.00 µg Dexamethasone/L (nominal), 0.91 µg Dexamethasone/L (mean measured)							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1	-	1.74E+07	-	3.36E+07	-	9.26E+06	4.742	-
2	-	1.56E+07	-	1.49E+07	-	2.14E+07	-	2.25E+06
3	-	2.77E+07	-	1.11E+07	2748	-	-	1.68E+06
4	-	3.79E+07	1453	-	-	9.07E+06	-	2.32E+06
5	-	1.96E+07	-	1.87E+07	3768	-	-	1.64E+04
6	-	1.18E+07	-	1.37E+07	558	-	-	1.89E+06
7	-	1.79E+07	-	5.44E+06	1400	-	-	3.12E+05
8	-	*>2,0e+08*	-	7.63E+06	4151	-	-	4.15E+06
9	-	1.19E+07	-	1.95E+07	-	2.59E+06	63.62	-
10	-	1.13E+07	-	2.12E+07	-	1.58E+06	59.44	-
11	-	5.00E+06	-	9.83E+06	-		14.64	-
12	293	-	358.1	-	-	2.39E+06	-	1.30E+06
13	4433	-	373.3	-	-	1.85E+06	-	6.54E+06
14	-	1.07E+07	92.22	-	9.393	-	-	5.63E+06
15	-	6.95E+06	-	7.76E+06	80.62	-	-	2.84E+06
16	-	2.27E+07	-	1.79E+07	2.421	-	-	4.73E+06
17	-	1.17E+07	-	2.25E+07	-	3.12E+06	-	7.05E+06(*)
18	-	2.59E+07	-	1.36E+07	11.92	-	6.484	-
19	1427	-	-	2.11E+07	-	9.85E+06	37.74	-
20	-	1.91E+05	-	7.16E+06	-	-	23.17	-
Geomean	**1228.37**	**1.14E+07**	**365.84**	**1.37E+07**	**208.16**	**4.64E+06**	**20.44**	**1.76E+06**

(*) Histological analysis did not confirm the sex of the respective fish.
The value highlighted in *italics* was not included in the calculation of the mean.

Table 189: F_1 generation, Vitellogenin content [ng/mL]; 3.20 µg Dexamethasone/L (nominal), respective 3.19 µg Dexamethasone/L (mean measured)

VTG [ng/mL]	3.20 µg Dexamethasone/L (nominal), 3.19 µg Dexamethasone/L (mean measured)							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1	-	1.48E+07	-	2.67E+07	11.69	-	-	6.08E+06
2	-	9.68E+06	19.15	-	990.8	-	-	4.09E+06
3	-	1.79E+07	-	3.72E+06	-	2.67E+07	-	1.71E+07
4	-	4.42E+07	36.86	-	-	7.49E+06	25.79	-
5	-	2.44E+07	-	2.96E+07	473	-	-	1.88E+07
6	-	6.81E+07	-	1.20E+07	43.42	-	-	1.32E+07
7	-	3.27E+07	-	3.65E+07	510.6	-	-	1.60E+07
8	-	1.45E+08	-	1.87E+07	29.14	-	140.3	-
9	-	2.18E+07	-	1.28E+07	12.7	-	-	9.00E+06
10	-	3.58E+06	668.5	-	32.72	-	-	2.13E+07
11	15.96	-	995.9	-	-	1.44E+07	-	1.31E+07
12	-	2.03E+07	1916	-	-	1.01E+07	-	5.71E+06
13	27.44	-	1195	-	-	1.37E+07	-	4.72E+06
14	19.15	-	2604	-	-	4.46E+06	-	1.09E+07
15	-	2.00E+07	-	5.11E+06	2295	-	12.66	-
16	20.35	-	-	9.73E+06	897	-	390.5	-
17	-	8.53E+06	-	5.19E+06	186.6	-	-	1.60E+07
18	-	1.69E+07	61.02		2003	-	-	8.08E+06
19	-	6.07E+06	-	1.88E+07	293.1	-	-	1.19E+07
20	-	-	24.81	-	-	-	-	4.63E+06
Geomean	**20.33**	**1.96E+07**	**252.97**	**1.28E+07**	**186.56**	**1.10E+07**	**65.03**	**9.93E+06**

Table 190: F$_1$ generation, Vitellogenin content [ng/mL], 10.0 µg Dexamethasone/L (nominal), respective 10.5 µg Dexamethasone/L (mean measured)

VTG [ng/mL]	10.0 µg Dexamethasone/L (nominal), 10.5 µg Dexamethasone/L (mean measured)							
Replicate	**A**		**B**		**C**		**D**	
Fish No	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**
1	-	2.19E+07	-	1.77E+07	14.71		-	2.76E+07
2	-	2.16E+07	10034	-	-	2.12E+07	-	1.19E+07
3	-	5.48E+06	-	3.62E+07	-	9.72E+06	-	9.91E+06
4	-	3.42E+07	-	4.05E+07	-	2.52E+07	-	2.28E+07
5	-	2.09E+07	-	2.37E+07	-	3.55E+07	55.84	-
6	-	2.14E+07	-	1.14E+07	47.05	-	71.93	-
7	3287	-	21.05	-	21.71	-	36.49	-
8	3.410	-	17.31	-	14.70	-	19.04	-
9	1437	-	25.56	-		1.93E+07	-	4.60E+07
10	-	7.93E+07	17.31	-	159.2	-	-	3.13E+07
11	-	4.85E+07	40.66	-	-	1.68E+07	-	3.40E+07
12	286.8	-	17.08	-	-	1.11E+07	-	7.53E+06
13	167.3	-	-	1.27E+07	-	1.76E+07	-	8.37E+06
14	38.10	-	-	2.04E+07	34.12	-	38.00	-
15	-	7.10E+06	15.80	-	-	2.59E+07	28.07	-
16	38.39	-	56.26	-	23.04	-	-	8.54E+06
17	126.7	-	-	2.03E+07	37.16	-	21.05	
18	45.22	-	7.773	-	-	5.59E+06	-	1.92E+07
19	40.31	-	33.49	-	11.38	-	40.82	
20	-	-	*<20*	-	-	-	-	7.25E+06
Geomean	**110.07**	**2.17E+07**	**21.95**	**2.09E+07**	**28.31**	**1.67E+07**	**35.56**	**1.60E+07**

The value highlighted in *italics* was not included in the calculation of the mean.

Table 191: F_1 generation, Vitellogenin content [ng/mL]; 32.0 µg Dexamethasone/L (nominal), respective 34.7 µg Dexamethasone/L (mean measured)

VTG [ng/mL]	32.0 µg Dexamethasone/L (nominal), 34.7 µg Dexamethasone/L (mean measured)							
Replicate	A		B		C		D	
Fish No	male	female	male	female	male	female	male	female
1	-	5.04E+07	-	2.88E+07	-	1.01E+07	-	2.58E+06
2	-	1.88E+07	-	1.88E+07	5.904	-	-	1.20E+07
3	961.5	-	-	1.60E+07	-	7.43E+06	-	4.94E+06
4	-	8.28E+06	-	6.81E+06	-	1.48E+07	-	7.00E+06
5	-	1.07E+07	-	5.62E+06	-	1.68E+06	*<10*	-
6	-	1.09E+07	-	3.94E+06	-	3.23E+06	*<10*	-
7	-	1.76E+07	-	2.32E+07	*<10*	-	-	5.28E+06
8	-	2.24E+06	-	5.29E+06	272.3	-	-	3.83E+06
9	-	3.30E+07	-	1.42E+07	8.810	-	8.989	-
10	-	1.28E+07	-	1.02E+07	562.7	-	-	5.71E+06
11	-	9.09E+06	36.21	-	11.14	-	9.976	-
12	-	1.24E+07	317.9	-	-	6.04E+06	-	3.57E+06
13	-	4.05E+06	18.93	-	-	9.91E+06	-	4.13E+06
14	-	3.53E+06	29.46	-	-	2.85E+06	-	2.42E+06
15	-	9.88E+06	-	5.80E+06	-	9.05E+06	-	2.65E+06
16	-	8.95E+06	11.92	-	11.14	-	-	1.14E+05
17	-	6.83E+06	-	3.60E+06	5.323	-	-	6.02E+06
18	12.89	-	-	7.05E+06	7.600	-	-	3.35E+06
19	-	6.16E+06	*<10*	-	7.205	-	-	2.42E+06
20	201.2	-	-	-	-	3.97E+06	-	1.72E+06
Geomean	**135.61**	**9.95E+06**	**37.74**	**9.21E+06**	**12.27**	**5.71E+06**	**9.47**	**3.18E+06**

Values highlighted in *italics* was not included in the calculation of the mean.

Table 192: F₁ generation, Vitellogenin content [ng/mL]; 100 µg Dexamethasone/L (nominal), respective 100 µg Dexamethasone/L (mean measured)

VTG [ng/mL]	100 µg Dexamethasone/L (nominal), 100 µg Dexamethasone/L (mean measured)							
Replicate	**A**		**B**		**C**		**D**	
Fish No	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**
1	-	9.17E+06	-	3.07E+06	-	3.50E+06	164	-
2	32.42	-	-	7.32E+06	*<20*	-	-	2.41E+06
3	21.17	-	-	9.02E+06	22.68	-	30.75	-
4	113.9	-	-	4.00E+06	-	2.80E+06	22.69	-
5	*<10*	-	-	5.89E+06	-	4.09E+06	-	1.11E+06
6	7.586	-	-	1.55E+06	-	2.02E+06	-	5.63E+05
7	-	7.91E+06	-	1.60E+05	-	4.19E+06	-	8.94E+05
8	-	3.81E+06	-	1.10E+04	44.61	-	-	2.02E+04
9	-	7.25E+06	-	1.13E+06	29.19	-	-	5.59E+05
10	4.745	-	-	8.66E+05	-	7.98E+06	-	1.86E+04
11	2.761	-	-	4.97E+06	352.1	-	-	5.71E+05
12	3.092	-	-	1.46E+06	-	1.53E+06	-	8.90E+05
13	*<10*	-	-	1.77E+06	-	4.16E+06	-	4.92E+05
14	-	3.09E+06	-	3.62E+06	-	5.83E+05	26.12	-
15	-	5.28E+06	-	5.16E+06	50.06	-	-	2.01E+06
16	-	8.53E+06	-	4.08E+06	-	8.45E+06	-	1.30E+06
17	206.1	-	10.71	-	-	2.68E+06	-	9.22E+05
18	-	1.25E+06	237.6	-	-	2.82E+06	19.24	-
19	-	-	-	1.74E+04	-	5.08E+05	-	1.66E+06
20	-	-	-	-	-	1.37E+06	-	4.64E+06
Geomean	**16.29**	**4.93E+06**	**50.44**	**1.37E+06**	**55.37**	**2.53E+06**	**35.64**	**6.43E+05**

Values highlighted in *italics* was not included in the calculation of the mean.

Table 193: F₁ generation, Vitellogenin content [ng/mL], summary

		Nominal concentration Dexamethasone [µg/L]						
		Control	**0.32**	**1.00**	**3.20**	**10.0**	**32.0**	**100**
		Mean measured concentration Dexamethasone [µg/L]						
	Replicate	**Control**	**0.33**	**0.91**	**3.19**	**10.5**	**34.7**	**100**
VTG males [ng/mL]	A	35.58	34.37	1228.37	20.33	110.07	135.61	16.29
	B	-(*)	35.93	365.84	252.97	21.95	37.74	50.44
	C	21.44	69.15	208.16	186.56	28.31	12.27	55.37
	D	27.07	26.27	20.44	65.03	35.56	9.47	35.64
	Mean	**28.03**	**41.43**	**455.70**	**131.22**	**48.97**	**48.77**	**39.44**
	SD	7.12	18.96	534.11	107.34	41.11	59.27	17.56
	RSD	25.4	45.8	117.2	81.8	83.9	121.5	44.5
VTG females [ng/mL]	A	1.23E+07	1.94E+06	1.14E+07	1.96E+07	2.17E+07	9.95E+06	4.93E+06
	B	-(*)	4.51E+06	1.37E+07	1.28E+07	2.09E+07	9.21E+06	1.37E+06
	C	4.72E+07	1.80E+07	4.64E+06	1.10E+07	1.67E+07	5.71E+06	2.53E+06
	D	2.32E+07	2.02E+07	1.76E+06	9.93E+06	1.60E+07	3.18E+06	6.43E+05
	Mean	**2.76E+07**	**1.11E+07**	**7.87E+06**	**1.33E+07**	**1.88E+07**	**7.01E+06**	**2.37E+06**
	SD	1.78E+07	9.25E+06	5.60E+06	4.36E+06	2.92E+06	3.15E+06	1.88E+06
	RSD	64.8	83.0	71.1	32.7	15.5	44.9	79.2

(*) Larvae of replicate B got lost due to a technical failure as an age of around 20 days was achieved. It was decided not to restart this group.

C.2.13 Histopathology of fish gonads, F_0 generation

Table 194: Histology raw data, F_0 generation

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
control	1	ZF1	0/1 1	f	f		2								
control	2	ZF2	0/1 2	m	m							2			
control	3	ZF3	0/1 3	f	f		3	1							
control	4	ZF4	0/1 4	f	f		2				1				
control	5	ZF5	0/1 5	m	m							3			
control	6	ZF6	0/1 6	f	f		3	1	1						
control	7	ZF7	0/1 7	m	m							2			
control	8	ZF8	0/1 8	m	m							2			
control	9	ZF9	0/1 9	f	f		3	1							
control	10	ZF10	0/1 10	m	m							2			
control	1	ZF11	0/2 1	f	f		3	2		1	1				
control	2	ZF12	0/2 2	m	m							2			
control	3	ZF13	0/2 3	f	f		3		1	1	3				

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
control	4	ZF14	0/2 4	m	m							3			
control	5	ZF15	0/2 5	f	f		3	1	2	1	3				
control	6	ZF16	0/2 6	m	m							2			
control	7	ZF17	0/2 7	f	f		2	1	1						
control	8	ZF18	0/2 8	m	m							2			
control	9	ZF19	0/2 9	f	f		3	1			1				
control	10	ZF20	0/2 10	m	m							2			
control	1	ZF21	0/3 1	m	m							2			
control	2	ZF22	0/3 2	m	m							2			
control	3	ZF23	0/3 3	f	f		2	1			2				
control	4	ZF24	0/3 4	m	m							2			
control	5	ZF25	0/3 5	m	m							3			
control	6	ZF26	0/3 6	f	f		4	2		2	1				
control	7	ZF27	0/3 7	m	m							2			
control	8	ZF28	0/3 8	f	f		3		2	1					
control	9	ZF29	0/3 9	f	f		2	2			2				

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
control	10	ZF30	0/3 10	f	f		3	2	1	1	2				
control	1	ZF31	0/4 1	m	m							2			
control	2	ZF32	0/4 2	f	f		3	2	2	1	2				
control	3	ZF33	0/4 3	m	m							2			
control	4	ZF34	0/4 4	f	f		3	1	1	1	2				
control	5	ZF35	0/4 5	m	m							2			
control	6	ZF36	0/4 6	m	m							2			
control	7	ZF37	0/4 7	f	f		3	2	1	1	2				
control	8	ZF38	0/4 8	m	m							2			
control	9	ZF39	0/4 9	f	f		2		1						
control	10	ZF40	0/4 10	f	f		3				1				
0.32	1	ZF41	1/1 1	f	f		3	2	1	1					
0.32	2	ZF42	1/1 2	f	f		3	2		1					
0.32	3	ZF43	1/1 3	f	f		2								
0.32	4	ZF44	1/1 4	f	f		3								
0.32	5	ZF45	1/1 5	f	f		3								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
0.32	6	ZF46	1/1 6	m	m							2			
0.32	7	ZF47	1/1 7	m	m							2			
0.32	8	ZF48	1/1 8	m	m							2	1		
0.32	9	ZF49	1/1 9	m	m							2			
0.32	10	ZF50	1/1 10	m	m							2			
0.32	1	ZF51	1/2 1	m	m							2			
0.32	2	ZF52	1/2 2	f	f		3	4	1	3					
0.32	3	ZF53	1/2 3	m	m							3			
0.32	4	ZF54	1/2 4	f	f		3	2	3	3	3				
0.32	5	ZF55	1/2 5	f	f		3	2	1	3	2				
0.32	6	ZF56	1/2 6	m	m							3			
0.32	7	ZF57	1/2 7	f	f		3	3	2	3					
0.32	8	ZF58	1/2 8	m	m							2			
0.32	9	ZF59	1/2 9	f	f		3	2	1	2					
0.32	10	ZF60	1/2 10	m	m							2			
0.32	1	ZF61	1/3 1	m	m							2			

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
0.32	2	ZF62	1/3 2	f	f		2	1	2	2	1				
0.32	3	ZF63	1/3 3	m	m							3			
0.32	4	ZF64	1/3 4	f	f		2	1		2					
0.32	5	ZF65	1/3 5	m	m							2			
0.32	6	ZF66	1/3 6	f	f		3	1	1	2	1				
0.32	7	ZF67	1/3 7	m	m							2		2	
0.32	8	ZF68	1/3 8	f	f		3	3	3	2	2				
0.32	9	ZF69	1/3 9	m	m							2			
0.32	10	ZF70	1/3 10	f	f		2								
0.32	1	ZF71	1/4 1	m	m							2			
0.32	2	ZF72	1/4 2	m								2			
0.32	3	ZF73	1/4 3	f	f		2	1	2	1	1				
0.32	4	ZF74	1/4 4	m	m							2			
0.32	5	ZF75	1/4 5	f	f		3	2	2	2	1				
0.32	6	ZF76	1/4 6	m	m							2			
0.32	7	ZF77	1/4 7	f	f		3	1	2	2					

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
0.32	8	ZF78	1/4 8	m	m							2			
0.32	9	ZF79	1/4 9	f	f		3	2	3	3					
0.32	10	ZF80	1/4 10	f	f		3								
1.00	1	ZF81	2/1 1	m	m							3			
1.00	2	ZF82	2/1 2	f	f		3								
1.00	3	ZF83	2/1 3	f	f		2	2	2	1	1				
1.00	4	ZF84	2/1 4	m	m							2			
1.00	5	ZF85	2/1 5	f	f		3	1	2	1					
1.00	6	ZF86	2/1 6	m	m							2			
1.00	7	ZF87	2/1 7	f	f		3	1	2	1	1				
1.00	8	ZF88	2/1 8	f	f		2	2	2	1	2				
1.00	9	ZF89	2/1 9	m	m							2			
1.00	10	ZF90	2/1 10	m	m							2		1	
1.00	1	ZF91	2/2 1	m	m							3			
1.00	2	ZF92	2/2 2	f	f		3	3	2	2	1				
1.00	3	ZF93	2/2 3	m	m							3			

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
1.00	4	ZF94	2/2 4	f	f		2								
1.00	5	ZF95	2/2 5	f	f		3		1		1				
1.00	6	ZF96	2/2 6	m	m							2			
1.00	7	ZF97	2/2 7	f	f		3	1	1	1					
1.00	8	ZF98	2/2 8	m	m							2			
1.00	9	ZF99	2/2 9	f	f		3	1	1	1	2				
1.00	10	ZF100	2/2 10	m	m							2			
1.00	1	ZF101	2/3 1	m	m							3			
1.00	2	ZF102	2/3 2	f	f		3								
1.00	3	ZF103	2/3 3	m	m							2			
1.00	4	ZF104	2/3 4	f	f		3	3	2	1					
1.00	5	ZF105	2/3 5	m	m							2			
1.00	6	ZF106	2/3 6	f	f		3								
1.00	7	ZF107	2/3 7	m	m							2			
1.00	8	ZF108	2/3 8	m	m							2			
1.00	9	ZF109	2/3 9	f	f		3		3	1					

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
1.00	10	ZF110	2/3 10	f	f		3								
1.00	1	ZF111	2/4 1	f	f		2	1	2	1	1				
1.00	2	ZF112	2/4 2	f	f		3	4	4	3					
1.00	3	ZF113	2/4 3	f	f		3								
1.00	4	ZF114	2/4 4	f	f		3	1	1	2					
1.00	5	ZF115	2/4 5	f	f		3	1							
1.00	6	ZF116	2/4 6	m	m							2			
1.00	7	ZF117	2/4 7	m	m							2			
1.00	8	ZF118	2/4 8	m	m							2			
1.00	9	ZF119	2/4 9	m	m							2			
1.00	10	ZF120	2/4 10	m	m							2			
3.20	1	ZF121	3/1 1	m	m							2			
3.20	2	ZF122	3/1 2	m	m							2			
3.20	3	ZF123	3/1 3	f	f		4	2	1	1	2				
3.20	4	ZF124	3/1 4	m	m							2			
3.20	5	ZF125	3/1 5	m	m							2			

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
3.20	6	ZF126	3/1 6	f	f		3								
3.20	7	ZF127	3/1 7	m	m							2			
3.20	8	ZF128	3/1 8	f	f		3	1	1						
3.20	9	ZF129	3/1 9	f	f		2				1				
3.20	10	ZF130	3/1 10	f	f		3	2	1		2				
3.20	1	ZF131	3/2 1	m	m							2			
3.20	2	ZF132	3/2 2	f	f		3	2	3	2	2				
3.20	3	ZF133	3/2 3	f	f		3	1	2	4					
3.20	4	ZF134	3/2 4	m	m							3			
3.20	5	ZF135	3/2 5	f	f		3	1	1	2					
3.20	6	ZF136	3/2 6	m	m							3			
3.20	7	ZF137	3/2 7	f	f		3								
3.20	8	ZF138	3/2 8	m	m							2			
3.20	9	ZF139	3/2 9	f	f		3		1	2	1				
3.20	10	ZF140	3/2 10	f	f		2	1	2		2				
3.20	1	ZF141	3/3 1	m	m							2			

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
3.20	2	ZF142	3/3 2	f	f		3								
3.20	3	ZF143	3/3 3	f	f		3								
3.20	4	ZF144	3/3 4	m	m							2			
3.20	5	ZF145	3/3 5	f	f		3		1						
3.20	6	ZF146	3/3 6	m	m							2			
3.20	7	ZF147	3/3 7	m	m							2			
3.20	8	ZF148	3/3 8	m	m							3			
3.20	9	ZF149	3/3 9	f	f		3								
3.20	10	ZF150	3/3 10	f	f		3	1							
3.20	1	ZF151	3/4 1	m	m							2			
3.20	2	ZF152	3/4 2	f	f		3	3	1	2	2				
3.20	3	ZF153	3/4 3	f	f		2	1	2	1	2				
3.20	4	ZF154	3/4 4	f	m							2			
3.20	5	ZF155	3/4 5	m	m							2			
3.20	6	ZF156	3/4 6	f	f		3								
3.20	7	ZF157	3/4 7	f	f		3	1	2	1					

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
3.20	8	ZF158	3/4 8	f	f		3		1	1					
3.20	9	ZF159	3/4 9	m	m							2			
3.20	10	ZF160	3/4 10	m	m							3			
10.0	1	ZF161	4/1 1	m	m							2			
10.0	2	ZF162	4/1 2	f	f		3								
10.0	3	ZF163	4/1 3	m	m							2		1	
10.0	4	ZF164	4/1 4	m	m							2			
10.0	5	ZF165	4/1 5	m	m							3			
10.0	6	ZF166	4/1 6	f	f		3			1					
10.0	7	ZF167	4/1 7	f	f		3		1	1	1				
10.0	8	ZF168	4/1 8	f	f		3	2	2	2	2				
10.0	9	ZF169	4/1 9	f	f		3								
10.0	10	ZF170	4/1 10	m	m							2			
10.0	1	ZF171	4/2 1	f	f		3	3	3	2	2				
10.0	2	ZF172	4/2 2	m	m							2			
10.0	3	ZF173	4/2 3	f	f		3			1					

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
10.0	4	ZF174	4/2 4	m	m							2			
10.0	5	ZF175	4/2 5	f	f		3			2					
10.0	6	ZF176	4/2 6	f	f		3	1		3					
10.0	7	ZF177	4/2 7	m	m							2			
10.0	8	ZF178	4/2 8	f	f		2				1				
10.0	9	ZF179	4/2 9	m	m							2			
10.0	10	ZF180	4/2 10	m	m							2			
10.0	1	ZF181	4/3 1	m	m							2			
10.0	2	ZF182	4/3 2	f	f		3			1	2				
10.0	3	ZF183	4/3 3	f	f		3								
10.0	4	ZF184	4/3 4	m	m							2			
10.0	5	ZF185	4/3 5	f	f		3	1	2	1	2				
10.0	6	ZF186	4/3 6	m	m							2			
10.0	7	ZF187	4/3 7	m	m							2			
10.0	8	ZF188	4/3 8	m	m							2			
10.0	9	ZF189	4/3 9	f	f		3	1	1	3	1				

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
10.0	10	ZF190	4/3 10	f	f		2			1					
10.0	1	ZF191	4/4 1	f	f		3								
10.0	2	ZF192	4/4 2	m	m							3			
10.0	3	ZF193	4/4 3	f	f		3	1		1					
10.0	4	ZF194	4/4 4	m	m							2			
10.0	5	ZF195	4/4 5	f	f		3		1	1					
10.0	6	ZF196	4/4 6	m	m							2			
10.0	7	ZF197	4/4 7	f	f		3								
10.0	8	ZF198	4/4 8	m	m							2			
10.0	9	ZF199	4/4 9	f	f		4			3					
10.0	10	ZF200	4/4 10	m	m							2			
32.0	1	ZF201	5/1 1	m	m							2			
32.0	2	ZF202	5/1 2	m	m							3			
32.0	3	ZF203	5/1 3	m	m							2			
32.0	4	ZF204	5/1 4	m	m							2			
32.0	5	ZF205	5/1 5	f	f		2		1	2	1				

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
32.0	6	ZF206	5/1 6	f	f		3	1	3	2					
32.0	7	ZF207	5/1 7	m	m							2			
32.0	8	ZF208	5/1 8	f	f		3								
32.0	9	ZF209	5/1 9	f	f		3			1					
32.0	1	ZF210	5/2 1	m	m							2			
32.0	2	ZF211	5/2 2	m	m							2			
32.0	3	ZF212	5/2 3	m	m							2			
32.0	4	ZF213	5/2 4	f	f		3		1	2					
32.0	5	ZF214	5/2 5	f	f		3		1						
32.0	6	ZF215	5/2 6	m	m							2			
32.0	7	ZF216	5/2 7	f	f		3		1		1				
32.0	8	ZF217	5/2 8	m	m							2			
32.0	9	ZF218	5/2 9	f	f		2	1	2		2				
32.0	10	ZF219	5/2 10	f	f		3								
32.0	1	ZF220	5/3 1	f	f		3		2	1	2				
32.0	2	ZF221	5/3 2	m	m							2			

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
32.0	3	ZF222	5/3 3	f	f		3								
32.0	4	ZF223	5/3 4	m	m							3			
32.0	5	ZF224	5/3 5	f	f		3								
32.0	6	ZF225	5/3 6	m	m							2			
32.0	7	ZF226	5/3 7	f	f		3								
32.0	8	ZF227	5/3 8	m	m							2			
32.0	9	ZF228	5/3 9	f	f		3								
32.0	10	ZF229	5/3 10	m	m							2			
32.0	1	ZF230	5/4 1	m	m							3			
32.0	2	ZF231	5/4 2	f	f		2		2		2				
32.0	3	ZF232	5/4 3	f	f		3	1	2	2					
32.0	4	ZF233	5/4 4	m	m							2			
32.0	5	ZF234	5/4 5	m	m							2			
32.0	6	ZF235	5/4 6	f	f		1		1	1					
32.0	7	ZF236	5/4 7	m	m							2			
32.0	8	ZF237	5/4 8	f	f		3	1	2	1					

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
32.0	9	ZF238	5/4 9	f	f		2				1				
32.0	10	ZF239	5/4 10	m	m							2			
100	1	ZF240	6/1 1	m	m							2			
100	2	ZF241	6/1 2	f	f		3								
100	3	ZF242	6/1 3	f	f		3	2	1	1					
100	4	ZF243	6/1 4	m	m							2		1	
100	5	ZF244	6/1 5	f	f		2								
100	6	ZF245	6/1 6	m	m							3			
100	7	ZF246	6/1 7	f	f		3								
100	8	ZF247	6/1 8	m	m							2			
100	9	ZF248	6/1 9	f	f		3								
100	10	ZF249	6/1 10	m	m							2			
100	1	ZF250	6/2 1	m	m							2			
100	2	ZF251	6/2 2	m	m							2			
100	3	ZF252	6/2 3	m	m							2			
100	4	ZF253	6/2 4	m	m							2			

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
100	5	ZF254	6/2 5	f	f		3								
100	6	ZF255	6/2 6	f	f		3								
100	7	ZF256	6/2 7	f	f		2				1				
100	8	ZF257	6/2 8	f	f		3	1							
100	9	ZF258	6/2 9	f	f		3								
100	10	ZF259	6/3 1	f	f		2	1	2						
100	2	ZF260	6/3 2	f	f		2	2	2	1	2				
100	3	ZF261	6/3 3	f	f		3								
100	4	ZF262	6/3 4	f	f		2	1							
100	5	ZF263	6/3 5	f	f		3	1			1				
100	6	ZF264	6/3 6	m	m							2		1	
100	7	ZF265	6/3 7	m	m							3			
100	8	ZF266	6/3 8	m	m							2		1	
100	9	ZF267	6/3 9	m	m							3			
100	10	ZF268	6/3 10	m	m							3			
100	1	ZF269	6/4 1	m	m							2			

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
100	2	ZF270	6/4 2	f	f		3								
100	3	ZF271	6/4 3	f	f		2		1						
100	4	ZF272	6/4 4	f	f		3								
100	5	ZF273	6/4 5	m	m							2			1
100	6	ZF274	6/4 6	f	f		2	2	2	1	2				
100	7	ZF275	6/4 7	m	m							3			
100	8	ZF276	6/4 8	f	f		2								
100	9	ZF277	6/4 9	m	m							2			
100	10	ZF278	6/4 10	m	m							2			

Table 195: F_0 generation, maturation stage, males; control, 0.32, 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.33, 0.91 and 3.19 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.13)

	Nominal concentration Dexamethasone [µg/L]															
	Control				0.32				1.00				3.20			
	Mean measured concentration Dexamethasone [µg/L]															
	Control				0.33				0.91				3.19			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Maturation stage, males**															
1			2	2		2	2	2	3	3	3		2	2	2	2
2	2	2	2					2					2			
3				2		3	3			3	2					
4		3	2					2	2				2	3	2	2
5	3		3	2			2				2		2			2
6		2		2	2	3		2	2	2		2		3	2	
7	2		2		2		2				2	2	2		2	
8	2	2		2	2	2		2		2	2	2		2	3	
9					2		2		2			2				2
10	2	2			2	2			2	2		2				3
Median	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.5**	**2.0**	**2.0**

Table 196: F_0 generation, maturation stage males; 10.0, 32.0 and 100 µg Dexamethasone/L (nominal), respective 10.5, 34.7 and 100 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.13)

	Nominal concentration Dexamethasone [µg/L]											
	10.0				**32.0**				**100**			
	Mean measured concentration Dexamethasone [µg/L]											
	10.5				**34.7**				**100**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Maturation stage, females**											
1	2		2		2	2		3	2	2		2
2		2		3	3	2	2			2		
3	2				2	2				2		
4	2	2	2	2	2		3	2	2	2		
5	3							2				2
6			2	2		2	2		3		2	
7		2	2		2			2			3	3
8			2	2		2	2		2		2	
9		2									3	2
10	2	2		2			2	2	2		3	2
Median	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**3.0**	**2.0**

Table 197: F_0 generation, maturation stage females; control, 0.32, 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.33, 0.91 and 3.19 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.13)

	Nominal concentration Dexamethasone [µg/L]															
	Control				**0.32**				**1.00**				**3.20**			
	Mean measured concentration Dexamethasone [µg/L]															
	Control				**0.33**				**0.91**				**3.19**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Maturation stage females**															
1	2	3			3							2				
2				3	3	3	2		3	3	3	3		3	3	3
3	3	3	2		2			2	2			3	4	3	3	2
4	2			3	3	3	2			2	3	3				
5		3			3	3		3	3	3		3		3	3	
6	3		4				3				3		3			3
7		2		3		3		3	3	3				3		3
8			3				3		2				3			3
9	3	3	2	2		3		3		3	3		2	3	3	
10			3	3			2	3			3		3	2	3	
Median	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	**2.0**	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**

Table 198: F_0 generation, maturation stage, females; 10.0, 32.0 and 100 µg Dexamethasone/L (nominal), respective 10.5, 34.7 and 100 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.13)

	Nominal concentration Dexamethasone [µg/L]											
	10.0				32.0				100			
	Mean measured concentration Dexamethasone [µg/L]											
	10.5				34.7				100			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Maturation stage, females											
1		3		3			3				2	
2	3		3					2	3		2	3
3		3	3	3			3	3	3		3	2
4						3					2	3
5		3	3	3	2	3	3		2	3	3	
6	3	3			3			1		3		2
7	3			3		3	3		3	2		
8	3	2			3			3		3		2
9	3		3	4	3	2	3	2	3	3		
10			2		-	3				-		
Median	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	**3.0**	**2.0**	**3.0**	**3.0**	**2.0**	**2.0**

Table 199: F_0 generation, maturation stage, summary

		Nominal concentration Dexamethasone [µg/L]						
		Control	0.32	1.00	3.20	10.0	32.0	100
		Mean measured concentration Dexamethasone [µg/L]						
		Control	0.33	0.91	3.19	10.5	34.7	100
Maturation stage, males, median value	A	2	2	2	2	2	2	2
	B	2	2	2	3	2	2	2
	C	2	2	2	2	2	2	3
	D	2	2	2	2	2	2	2
Maturation stage, females, median value	A	3	3	3	3	3	3	3
	B	3	3	3	3	3	3	3
	C	3	2	3	3	3	3	2
	D	3	3	3	3	3	2	2

Table 200: F_0, increased proportion of spermatogonia, males; control, 0.32, 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.33, 0.91 and 3.19 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.13)

	Nominal concentration Dexamethasone [µg/L]															
	Control				0.32				1.00				3.20			
	Mean measured concentration Dexamethasone [µg/L]															
	Control				0.33				0.91				3.19			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Increased proportion of spermatogonia, males															
1	-	-	0	0	-	0	0	0	0	0	0	-	0	0	0	0
2	0	0	0	-	-	-	-	0	-	-	-	-	0	-	-	-
3	-	-	-	0	-	0	0	-	-	0	0	-	-	-	-	-
4	-	0	0	-	-	-	-	0	0	-	-	-	0	0	0	0
5	0	-	0	0	-	-	0	-	-	-	0	-	0	-	-	0
6	-	0	-	0	0	0	-	0	0	0	-	0	-	0	0	-
7	0	-	0	-	0	-	2	-	-	-	0	0	0	-	0	-
8	0	0	-	0	0	0	-	0	-	0	0	0	-	0	0	-
9	-	-	-	-	0	-	0	-	0	-	-	0	-	-	-	0
10	0	0	-	-	0	0	-	-	1	0	-	0	-	-	-	0
Median	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Table 201: F_0, increased proportion of spermatogonia, males; 10.0, 32.0 and 100 µg Dexamethasone/L (nominal), respective 10.5, 34.7 and 100 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.13)

	Nominal concentration Dexamethasone [µg/L]											
	10.0				32.0				100			
	Mean measured concentration Dexamethasone [µg/L]											
	10.5				34.7				100			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Increased proportion of spermatogonia, males											
1	0	-	0	-	0	0	-	0	0	0	-	0
2	-	0	-	0	0	0	0	-	-	0	-	-
3	1	-	-	-	0	0	-	-	-	0	-	-
4	0	0	0	0	0	-	0	0	1	0	-	-
5	0	-	-	-	-	-	-	0	-	-	-	0
6	-	-	0	0	-	0	0	-	0	-	1	-
7	-	0	0	-	0	-	-	0	-	-	0	0
8	-	-	0	0	-	0	0	-	0	-	1	-
9	-	0	-	-	-	-	-	-	-	-	0	0
10	0	0	-	0	-	-	0	0	0	-	0	0
Median	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Table 202: F_0, increased oocyte atresia, females; control, 0.32, 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.33, 0.91 and 3.19 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.13)

	Nominal concentration Dexamethasone [µg/L]															
	Control				0.32				1.00				3.20			
	Mean measured concentration Dexamethasone [µg/L]															
	Control				0.33				0.91				3.19			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Increased oocyte atresia, females															
1	0	2	-	-	2	-	-	-	-	-	-	1	-	-	-	-
2	-	-	-	2	2	4	1	-	0	3	0	4	-	2	0	3
3	1	0	1	-	0	-	-	1	2	-	-	0	2	1	0	1
4	0	-	-	1	0	2	1	-	-	0	3	1	-	-	-	-
5	-	1	-	-	0	2	-	2	1	0	-	1	-	1	0	-
6	1	-	2	-	-	-	1	-	-	-	0	-	0	-	-	0
7	-	1	-	2	-	3	-	1	1	1	-	-	-	0	-	1
8	-	-	0	-	-	-	3	-	2	-	-	-	1	-	-	0
9	1	1	2	0	-	2	-	2	-	1	0	-	0	0	0	-
10	-	-	2	0	-	-	0	0	-	-	0	-	2	1	1	-
Median	**1.0**	**1.0**	**2.0**	**1.0**	**0.0**	**2.0**	**1.0**	**1.0**	**1.0**	**1.0**	**0.0**	**1.0**	**1.0**	**1.0**	**0.0**	**1.0**

Table 203: F_0, increased oocyte atresia, females; 10.0, 32.0 and 100 µg Dexamethasone/L (nominal), respective 10.5, 34.7 and 100 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.13)

	Nominal concentration Dexamethasone [µg/L]											
	10.0				32.0				100			
	Mean measured concentration Dexamethasone [µg/L]											
	10.5				34.7				100			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Increased oocyte atresia, females**											
1	-	3	-	0	-	-	0	-	-	-	1	-
2	0	-	0	-	-	-	-	0	0	-	2	0
3	-	0	0	1	-	-	0	1	2	-	0	0
4	-	-	-	-	-	0	-	-	-	-	1	0
5	-	0	1	0	0	0	0	-	0	0	1	-
6	0	1	-	-	1	-	-	0	-	0	-	2
7	0	-	-	0	-	0	0	-	0	0	-	-
8	2	0	-	-	0	-	-	1	-	1	-	0
9	0	-	1	0	0	1	0	0	0	0	-	-
10	-	-	0	-	-	0	-	-	-	-	-	-
Median	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**1.0**	**0.0**

Table 204: F_0 generation, egg debris, females; control, 0.32, 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.33, 0.91 and 3.19 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.13)

	Nominal concentration Dexamethasone [µg/L]															
	Control				0.32				1.00				3.20			
	Mean measured concentration Dexamethasone [µg/L]															
	Control				0.33				0.91				3.19			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Egg debris, females**															
1	0	0	-	-	1	-	-	-	-	-	-	2	-	-	-	-
2	-	-	-	2	0	1	2	-	0	2	0	4	-	3	0	1
3	0	1	0	-	0	-	-	2	2	-	-	0	1	2	0	2
4	0	-	-	1	0	3	0	-	-	0	2	1	-	-	-	-
5	-	2	-	-	0	1	-	2	2	1	-	0	-	1	1	-
6	1	-	0	-	-	-	1	-	-	-	0	-	0	-	-	0
7	-	1	-	1	-	2	-	2	2	1	-	-	-	0	-	2
8	-	-	2	-	-	-	3	-	2	-	-	-	1	-	-	1
9	0	0	0	1	-	1	-	3	-	1	3	-	0	1	0	-
10	-	-	1	0	-	-	0	0	-	-	0	-	1	2	0	-
Median	**0.0**	**1.0**	**0.0**	**1.0**	**0.0**	**1.0**	**1.0**	**2.0**	**2.0**	**1.0**	**0.0**	**1.0**	**1.0**	**1.5**	**0.0**	**1.0**

Table 205: F_0, egg debris, females; 10.0, 32.0 and 100 µg Dexamethasone/L (nominal), respective 10.5, 34.7 and 100 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.13)

	Nominal concentration Dexamethasone [µg/L]											
	10.0				**32.0**				**100**			
	Mean measured concentration Dexamethasone [µg/L]											
	10.5				**34.7**				**100**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Egg debris, females**											
1	-	3	-	0	-	-	2	-	-	-	2	-
2	0	-	0	-	-	-	-	2	0	-	2	0
3	-	0	0	0	-	-	0	2	1	-	0	1
4	-	-	-	-	-	1	-	-	-	-	0	0
5	-	0	2	1	1	1	0	-	0	0	0	-
6	0	0	-	-	3	-	-	1	-	0	-	2
7	1	-	-	0	-	1	0	-	0	0	-	-
8	2	0	-	-	0	-	-	2	-	0	-	0
9	0	-	1	0	0	2	0	0	0	0	-	-
10	-	-	0	-	-	0	-	-	-	-	-	-
Median	**0.0**	**0.0**	**0.0**	**0.0**	**0.5**	**1.0**	**0.0**	**2.0**	**0.0**	**0.0**	**0.0**	**0.0**

Table 206: F_0, granulomatous inflammation, females; control, 0.32, 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.33, 0.91 and 3.19 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.13)

	Nominal concentration Dexamethasone [µg/L]															
	Control				0.32				1.00				3.20			
	Mean measured concentration Dexamethasone [µg/L]															
	Control				0.33				0.91				3.19			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Granulomatous inflammation, females															
1	0	1	-	-	1	-	-	-	-	-	-	1	-	-	-	-
2	-	-	-	1	1	3	2	-	0	2	0	3	-	2	0	2
3	0	1	0	-	0	-	-	1	1	-	-	0	1	4	0	1
4	0	-	-	1	0	3	2	-	-	0	1	2	-	-	-	-
5	-	1	-	-	0	3	-	2	1	0	-	0	-	2	0	-
6	0	-	2	-	-	-	2	-	-	-	0	-	0	-	-	0
7	-	0	-	1	-	3	-	2	1	1	-	-	-	0	-	1
8	-	-	1	-	-	-	2	-	1	-	-	-	0	-	-	1
9	0	0	0	0	-	2	-	3	-	1	1	-	0	2	0	-
10	-	-	1	0	-	-	0	0	-	-	0	-	0	0	0	-
Median	**0.0**	**1.0**	**1.0**	**1.0**	**0.0**	**3.0**	**2.0**	**2.0**	**1.0**	**1.0**	**0.0**	**1.0**	**0.0**	**2.0**	**0.0**	**1.0**

Table 207: F_0, granulomatous inflammation, females; 10.0, 32.0 and 100 µg Dexamethasone/L (nominal), respective 10.5, 34.7 and 100 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.13)

	Nominal concentration Dexamethasone [µg/L]											
	10.0				32.0				100			
	Mean measured concentration Dexamethasone [µg/L]											
	10.5				34.7				100			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Granulomatous inflammation, females**											
1	-	2	-	0	-	-	1	-	-	-	0	-
2	0	-	1	-	-	-	-	0	0	-	1	0
3	-	1	0	1	-	-	0	2	1	-	0	0
4	-	-	-	-	-	2	-	-	-	-	0	0
5	-	2	1	1	2	0	0	-	0	0	0	-
6	1	3	-	-	2	-	-	1	-	0	-	1
7	1	-	-	0	-	0	0	-	0	0	-	-
8	2	0	-	-	0	-	-	1	-	0	-	0
9	0	-	3	3	1	0	0	0	0	0	-	-
10	-	-	1	-	-	0	-	-	-	-	-	-
Median	**1.0**	**2.0**	**1.0**	**1.0**	**1.5**	**0.0**	**0.0**	**1.0**	**0.0**	**0.0**	**0.0**	**0.0**

Table 208: F_0, increased post-ovulatory follicles, females; control, 0.32, 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.33, 0.91 and 3.19 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.13)

	Nominal concentration Dexamethasone [µg/L]															
	Control				0.32				1.00				3.20			
	Mean measured concentration Dexamethasone [µg/L]															
	Control				0.33				0.91				3.19			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Increased post-ovulatory follicles, females**															
1	0	1	-	-	0	-	-	-	-	-	-	1	-	-	-	-
2	-	-	-	2	0	0	1	-	0	1	0	0	-	2	0	2
3	0	3	2	-	0	-	-	1	1	-	-	0	2	0	0	2
4	1	-	-	2	0	3	0	-	-	0	0	0	-	-	-	-
5	-	3	-	-	0	2	-	1	0	1	-	0	-	0	0	-
6	0	-	1	-	-	-	1	-	-	-	0	-	0	-	-	0
7	-	0	-	2	-	0	-	0	1	0	-	-	-	0	-	0
8	-	-	0	-	-	-	2	-	2	-	-	-	0	-	-	0
9	0	1	2	0	-	0	-	0	-	2	0	-	1	1	0	-
10	-	-	2	1	-	-	0	0	-	-	0	-	2	2	0	-
Median	**0.0**	**1.0**	**2.0**	**2.0**	**0.0**	**0.0**	**1.0**	**0.0**	**1.0**	**1.0**	**0.0**	**0.0**	**1.0**	**0.5**	**0.0**	**0.0**

Table 209: F_0, increased post-ovulatory follicles, females; 10.0, 32.0 and 100 µg Dexamethasone/L (nominal), respective 10.5, 34.7 and 100 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.13)

	Nominal concentration Dexamethasone [µg/L]											
	10.0				32.0				100			
	Mean measured concentration Dexamethasone [µg/L]											
	10.5				34.7				100			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Increased post-ovulatory follicles, females											
1	-	2	-	0	-	-	2	-	-	-	0	-
2	0	-	2	-	-	-	-	2	0	-	2	0
3	-	0	0	0	-	-	0	0	0	-	0	0
4	-	-	-	-	-	0	-	-	-	-	0	0
5	-	0	2	0	1	0	0	-	0	0	1	-
6	0	0	-	-	0	-	-	0	-	0	-	2
7	1	-	-	0	-	1	0	-	0	1	-	-
8	2	1	-	-	0	-	-	0	-	0	-	0
9	0	-	1	0	0	2	0	1	0	0	-	-
10	-	-	0	-	-	0	-	-	-	-	-	-
Median	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 210: F_0 generation, grading of lesions severity, summary

		Nominal concentration Dexamethasone [µg/L]						
		Control	**0.32**	**1.00**	**3.20**	**10.0**	**32.0**	**100**
		Mean measured concentration Dexamethasone [µg/L]						
	Replicate	**Control**	**0.33**	**0.91**	**3.19**	**10.5**	**34.7**	**100**
Increased proportion of spermatogonia, males, median value	A	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	B	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	C	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Increased oocyte atresia, females, median value	A	1.0	0.0	1.0	1.0	0.0	0.0	0.0
	B	1.0	2.0	1.0	1.0	0.0	0.0	0.0
	C	2.0	1.0	0.0	0.0	0.0	0.0	1.0
	D	1.0	1.0	1.0	1.0	0.0	0.0	0.0
Egg debris, females, median value	A	0.0	0.0	2.0	1.0	0.0	0.5	0.0
	B	1.0	1.0	1.0	1.5	0.0	1.0	0.0
	C	0.0	1.0	0.0	0.0	0.0	0.0	0.0
	D	1.0	2.0	1.0	1.0	0.0	2.0	0.0
Granumatomous inflammation, females, median value	A	0.0	0.0	1.0	0.0	1.0	1.5	0.0
	B	1.0	3.0	1.0	2.0	2.0	0.0	0.0
	C	1.0	2.0	0.0	0.0	1.0	0.0	0.0
	D	1.0	2.0	1.0	1.0	1.0	1.0	0.0
Increased post-ovulatory follicles, females, median value	A	0.0	0.0	1.0	1.0	0.0	0.0	0.0
	B	1.0	0.0	1.0	0.5	0.0	0.0	0.0
	C	2.0	1.0	0.0	0.0	1.0	0.0	0.0
	D	2.0	0.0	0.0	0.0	0.0	0.0	0.0

C.2.14 Histopathology of fish gonads, F_1 generation

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
control	1	ZF1	0/1 1	m	m							2			
control	2	ZF2	0/1 2	m	m							2			
control	3	ZF3	0/1 3	f	f		2								
control	4	ZF4	0/1 4	m	m							2			
control	5	ZF5	0/1 5	f	f		2								
control	6	ZF6	0/1 6	f	f		2								
control	7	ZF7	0/1 7	m	m							2			
control	8	ZF8	0/1 8	m	m							2			
control	9	ZF9	0/1 9	m	m							2			
control	10	ZF10	0/1 10	f	f		3								
control	11	ZF11	0/1 11	f	f		3								
control	12	ZF12	0/1 12	f	f		2								
control	13	ZF13	0/1 13	f	f		2		1						
control	14	ZF14	0/1 14	f	f		2								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
control	15	ZF15	0/1 15	m	m							2			
control	16	ZF16	0/1 16	m	m							2			
control	17	ZF17	0/1 17	f	f		3								
control	18	ZF18	0/1 18	f	f		3		1						
control	19	ZF19	0/1 19	m	m							2			
control	20	ZF20	0/1 20	m	m							2			
control	1	ZF21	0/3 1	f	f		2		1						
control	2	ZF22	0/3 2	f	f		2	1	2						
control	3	ZF23	0/3 3	f	f		2				1				
control	4	ZF24	0/3 4	m	m							2			
control	5	ZF25	0/3 5	f	f		2				1				
control	6	ZF26	0/3 6	f	f		2				1				
control	7	ZF27	0/3 7	f	f		2								
control	8	ZF28	0/3 8	m	m							2			
control	9	ZF29	0/3 9	m	m							2			
control	10	ZF30	0/3 10	m	m							2	1		

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
control	11	ZF31	0/3 11	f	f		2	1							
control	12	ZF32	0/3 12	m	m							2			
control	13	ZF33	0/3 13	m	m							2			
control	14	ZF34	0/3 14	m	m							2			
control	15	ZF35	0/3 15	m	m							2			
control	16	ZF36	0/3 16	f	f		3								
control	17	ZF37	0/3 17	f	f		2								
control	18	ZF38	0/3 18	f	f		3			1					
control	19	ZF39	0/3 19	f	f		2								
control	20	ZF40	0/3 20	m	m							2			
control	1	ZF41	0/4 1	f	f		2								
control	2	ZF42	0/4 2	f	f		2								
control	3	ZF43	0/4 3	f	f		2								
control	4	ZF44	0/4 4	f	f		3								
control	5	ZF45	0/4 5	m	m							2			
control	6	ZF46	0/4 6	m	m							2			

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
control	7	ZF47	0/4 7	m	m							2			
control	8	ZF48	0/4 8	f	f		3								
control	9	ZF49	0/4 9	f	f		2	1	1	2					
control	10	ZF50	0/4 10	f	f		3								
control	11	ZF51	0/4 11	f	f		2								
control	12	ZF52	0/4 12	f	f		2								
control	13	ZF53	0/4 13	m	m							2		1	
control	14	ZF54	0/4 14	m	m							2			
control	15	ZF55	0/4 15	f	f		2		1						
control	16	ZF56	0/4 16	f	f		2								
control	17	ZF57	0/4 17	f	f		2			1					
control	18	ZF58	0/4 18	m	m							2			
control	19	ZF59	0/4 19	m	m							2			
control	20	ZF60	0/4 20	f	f		2		1						
0.32	1	ZF61	1/1 1	f	f		3			1					
0.32	2	ZF62	1/1 2	f	f		2								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
0.32	3	ZF63	1/1 3	f	f		2								
0.32	4	ZF64	1/1 4	f	f		3								
0.32	5	ZF65	1/1 5	f	f		2	1	1	1					
0.32	6	ZF66	1/1 6	m	m							2			
0.32	7	ZF67	1/1 7	f	f		2			1					
0.32	8	ZF68	1/1 8	f	f		2				1				
0.32	9	ZF69	1/1 9	m	m							2			
0.32	10	ZF70	1/1 10	m	m							3			
0.32	11	ZF71	1/1 11	m	m							2	1		
0.32	12	ZF72	1/1 12	m	m							2			
0.32	13	ZF73	1/1 13	f	f		2								
0.32	14	ZF74	1/1 14	m	m							2			
0.32	15	ZF75	1/1 15	f	f		3	2							
0.32	16	ZF76	1/1 16	f	f		2				1				
0.32	17	ZF77	1/1 17	f	f		2								
0.32	18	ZF78	1/1 18	m	m							2			

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
0.32	19	ZF79	1/1 19	m	m							3			
0.32	20	ZF80	1/1 20	m	m							2			
0.32	1	ZF81	1/2 1	f	f		3			1					
0.32	2	ZF82	1/2 2	f	f		2								
0.32	3	ZF83	1/2 3	f	f		3								
0.32	4	ZF84	1/2 4	f	f		2								
0.32	5	ZF85	1/2 5	f	f		3								
0.32	6	ZF86	1/2 6	f	f		2								
0.32	7	ZF87	1/2 7	m	m							2			
0.32	8	ZF88	1/2 8	f	f		2								
0.32	9	ZF89	1/2 9	m	m							2			
0.32	10	ZF90	1/2 10	m	m							2			
0.32	11	ZF91	1/2 11	m	m							2			
0.32	12	ZF92	1/2 12	f	f		3								
0.32	13	ZF93	1/2 13	f	f		3								
0.32	14	ZF94	1/2 14	m	m							2			

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
0.32	15	ZF95	1/2 15	f	f		2								
0.32	16	ZF96	1/2 16	f	f		3								
0.32	17	ZF97	1/2 17	f	f		3	1	1						
0.32	18	ZF98	1/2 18	f	f		3								
0.32	19	ZF99	1/2 19	m	m							2			
0.32	20	ZF100	1/2 20	m	m							2	1		
0.32	1	ZF101	1/3 1	f	f		2		1	1					
0.32	2	ZF102	1/3 2	f	f		2		1						
0.32	3	ZF103	1/3 3	f	f		2			1					
0.32	4	ZF104	1/3 4	f	f		3								
0.32	5	ZF105	1/3 5	m	m							2			
0.32	6	ZF106	1/3 6	m	m							1			
0.32	7	ZF107	1/3 7	m	m							2			
0.32	8	ZF108	1/3 8	m	m							2			
0.32	9	ZF109	1/3 9	f	f		2	1	1						
0.32	10	ZF110	1/3 10	f	f		3		1						

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
0.32	11	ZF111	1/3 11	f	f		2								
0.32	12	ZF112	1/3 12	f	f		2			1					
0.32	13	ZF113	1/3 13	f	f		2			1	1				
0.32	14	ZF114	1/3 14	m	m							2			
0.32	15	ZF115	1/3 15	m	m							2			
0.32	16	ZF116	1/3 16	f	f		2				1				
0.32	17	ZF117	1/3 17	f	f		2								
0.32	18	ZF118	1/3 18	m	m							2			
0.32	19	ZF119	1/3 19	f	f		2								
0.32	20	ZF120	1/3 20	f	f		2	1	1	1					
0.32	1	ZF121	1/4 1	m	m							2	1		
0.32	2	ZF122	1/4 2	f	f		2				1				
0.32	3	ZF123	1/4 3	f	f		2								
0.32	4	ZF124	1/4 4	f	f		2	1			1				
0.32	5	ZF125	1/4 5	f	f		2								
0.32	6	ZF126	1/4 6	f	f		2								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
0.32	7	ZF127	1/4 7	f	f		3								
0.32	8	ZF128	1/4 8	f	f		2								
0.32	9	ZF129	1/4 9	f	f		2								
0.32	10	ZF130	1/4 10	m	m							2			
0.32	11	ZF131	1/4 11	f	f		2								
0.32	12	ZF132	1/4 12	f	f		2								
0.32	13	ZF133	1/4 13	f	f		2	1							
0.32	14	ZF134	1/4 14	f	f		2								
0.32	15	ZF135	1/4 15	f	f		2		1						
0.32	16	ZF136	1/4 16	f	f		2				1				
0.32	17	ZF137	1/4 17	m	m							2			
0.32	18	ZF138	1/4 18	f	f		2	1			1				
0.32	19	ZF139	1/4 19	m	m							2			
0.32	20	ZF140	1/4 20	m	m							2			
1.00	1	ZF141	2/1 1	f	f		3								
1.00	2	ZF142	2/1 2	f	f		2								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
1.00	3	ZF143	2/1 3	f	f		2				1				
1.00	4	ZF144	2/1 4	f	f		3								
1.00	5	ZF145	2/1 5	f	f		2								
1.00	6	ZF146	2/1 6	f	f		2								
1.00	7	ZF147	2/1 7	f	f		3		1						
1.00	8	ZF148	2/1 8	f	f		2				1				
1.00	9	ZF149	2/1 9	f	f		2								
1.00	10	ZF150	2/1 10	f	f		2								
1.00	11	ZF151	2/1 11	f	f		2			1	2				
1.00	12	ZF152	2/1 12	m	m							2			
1.00	13	ZF153	2/1 13	m	m							2			
1.00	14	ZF154	2/1 14	f	f		2			1					
1.00	15	ZF155	2/1 15	f	f		3								
1.00	16	ZF156	2/1 16	f	f		2								
1.00	17	ZF157	2/1 17	f	f		1			1					
1.00	18	ZF158	2/1 18	f	f		2								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
1.00	19	ZF159	2/1 19	m	m							2			
1.00	20	ZF160	2/1 20	f	f		2	2	1	1	2				
1.00	1	ZF161	2/2 1	f	f		3								
1.00	2	ZF162	2/2 2	f	f		2								
1.00	3	ZF163	2/2 3	f	f		2	1	2	1	2				
1.00	4	ZF164	2/2 4	m	m							2			
1.00	5	ZF165	2/2 5	f	f		3								
1.00	6	ZF166	2/2 6	f	f		2								
1.00	7	ZF167	2/2 7	f	f		2			1					
1.00	8	ZF168	2/2 8	f	f		2				1				
1.00	9	ZF169	2/2 9	f	f		3								
1.00	10	ZF170	2/2 10	f	f		3								
1.00	11	ZF171	2/2 11	f	f		2								
1.00	12	ZF172	2/2 12	m	m							2			
1.00	13	ZF173	2/2 13	m	m							2	1		
1.00	14	ZF174	2/2 14	m	m							2			

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
1.00	15	ZF175	2/2 15	f	f		2								
1.00	16	ZF176	2/2 16	f	f		3								
1.00	17	ZF177	2/2 17	f	f		2		1						
1.00	18	ZF178	2/2 18	f	f		2		2		1				
1.00	19	ZF179	2/2 19	f	f		2								
1.00	20	ZF180	2/2 20	f	f		2								
1.00	1	ZF181	2/3 1	f	f		3		1	1					
1.00	2	ZF182	2/3 2	f	f		2								
1.00	3	ZF183	2/3 3	m	m							3			
1.00	4	ZF184	2/3 4	f	f		2								
1.00	5	ZF185	2/3 5	m	m							2			
1.00	6	ZF186	2/3 6	m	m							2			
1.00	7	ZF187	2/3 7	m	m							2			
1.00	8	ZF188	2/3 8	m	m							2			
1.00	9	ZF189	2/3 9	f	f		3								
1.00	10	ZF190	2/3 10	f	f		2								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
1.00	11	ZF191	2/3 11	f	f		2								
1.00	12	ZF192	2/3 12	f	f		2	1							
1.00	13	ZF193	2/3 13	f	f		2								
1.00	14	ZF194	2/3 14	m	m							2			
1.00	15	ZF195	2/3 15	m	m							2			
1.00	16	ZF196	2/3 16	m	m							2			
1.00	17	ZF197	2/3 17	f	f		2								
1.00	18	ZF198	2/3 18	m	m							2			
1.00	19	ZF199	2/3 19	f	f		1								
1.00	1	ZF200	2/4 1	m	m							2			
1.00	2	ZF201	2/4 2	f	f		2								
1.00	3	ZF202	2/4 3	f	f		2								
1.00	4	ZF203	2/4 4	f	f		2								
1.00	5	ZF204	2/4 5	f	f		2			1					
1.00	6	ZF205	2/4 6	f	f		2								
1.00	7	ZF206	2/4 7	f	f		2								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
1.00	8	ZF207	2/4 8	f	f		2								
1.00	9	ZF208	2/4 9	m	m							2			
1.00	10	ZF209	2/4 10	m	m							2			1
1.00	11	ZF210	2/4 11	m	m							2			
1.00	12	ZF211	2/4 12	f	f		2								
1.00	13	ZF212	2/4 13	f	f		3								
1.00	14	ZF213	2/4 14	f	f		2								
1.00	15	ZF214	2/4 15	f	f		2								
1.00	16	ZF215	2/4 16	f	f		2								
1.00	17	ZF216	2/4 17	f	f		3								
1.00	18	ZF217	2/4 18	m	m							2			
1.00	19	ZF218	2/4 19	m	m							2			
1.00	20	ZF219	2/4 20	m	m							2			
3.20	1	ZF220	3/1 1	f	f		2			2					
3.20	2	ZF221	3/1 2	f	f		2								
3.20	3	ZF222	3/1 3	f	f		3								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
3.20	4	ZF223	3/1 4	f	f		2								
3.20	5	ZF224	3/1 5	f	f		3								
3.20	6	ZF225	3/1 6	f	f		2		1		1				
3.20	7	ZF226	3/1 7	f	f		3								
3.20	8	ZF227	3/1 8	f	f		2			1	1				
3.20	9	ZF228	3/1 9	f	f		2			1					
3.20	10	ZF229	3/1 10	f	f		3								
3.20	11	ZF230	3/1 11	m	m							2			
3.20	12	ZF231	3/1 12	f	f		2		1						
3.20	13	ZF232	3/1 13	m	m							2			
3.20	14	ZF233	3/1 14	m	m							2			
3.20	15	ZF234	3/1 15	f	f		2								
3.20	16	ZF235	3/1 16	m	m							2			
3.20	17	ZF236	3/1 17	f	f		3								
3.20	18	ZF237	3/1 18	f	f		2								
3.20	19	ZF238	3/1 19	f	f		3	1	1		1				

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
3.20	1	ZF239	3/2 1	f	f		2								
3.20	2	ZF240	3/2 2	m	m							2			
3.20	3	ZF241	3/2 3	f	f		2								
3.20	4	ZF242	3/2 4	m	m							2			
3.20	5	ZF243	3/2 5	f	f		3								
3.20	6	ZF244	3/2 6	f	f		2								
3.20	7	ZF245	3/2 7	f	f		3		1						
3.20	8	ZF246	3/2 8	f	f		2								
3.20	9	ZF247	3/2 9	f	f		2								
3.20	10	ZF248	3/2 10	m	m							2			
3.20	11	ZF249	3/2 11	m	m							2			
3.20	12	ZF250	3/2 12	m	m							2			
3.20	13	ZF251	3/2 13	m	m							2			
3.20	14	ZF252	3/2 14	m	m							2			
3.20	15	ZF253	3/2 15	f	f		2								
3.20	16	ZF254	3/2 16	f	f		3			3					

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
3.20	17	ZF255	3/2 17	f	f		2								
3.20	18	ZF256	3/2 18	m	m							2			
3.20	19	ZF257	3/2 19	f	f		2		1		3				
3.20	20	ZF258	3/2 20	m	m							2			
3.20	1	ZF259	3/3 1	m	m							2			
3.20	2	ZF260	3/3 2	m	m							2			
3.20	3	ZF261	3/3 3	f	f		2								
3.20	4	ZF262	3/3 4	f	f		2			1					
3.20	5	ZF263	3/3 5	m	m							3			
3.20	6	ZF264	3/3 6	m	m							2			
3.20	7	ZF265	3/3 7	m	m							2			
3.20	8	ZF266	3/3 8	m	m							2			
3.20	9	ZF267	3/3 9	m	m							2			
3.20	10	ZF268	3/3 10	m	m							1	4		
3.20	11	ZF269	3/3 11	f	f		3								
3.20	12	ZF270	3/3 12	f	f		2								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
3.20	13	ZF271	3/3 13	f	f		2	1							
3.20	14	ZF272	3/3 14	f	f		2		2	1	3				
3.20	15	ZF273	3/3 15	m	m							2			
3.20	16	ZF274	3/3 16	m	m							2			
3.20	17	ZF275	3/3 17	m	m							2			
3.20	18	ZF276	3/3 18	m	m							2			
3.20	19	ZF277	3/3 19	m	m							2			
3.20	1	ZF278	3/4 1	f	f		2								
3.20	2	ZF279	3/4 2	f	f		2								
3.20	3	ZF280	3/4 3	f	f		2								
3.20	4	ZF281	3/4 4	m	m							2			
3.20	5	ZF282	3/4 5	f	f		2								
3.20	6	ZF283	3/4 6	f	f		3								
3.20	7	ZF284	3/4 7	f	f		2								
3.20	8	ZF285	3/4 8	m	m							2			
3.20	9	ZF286	3/4 9	f	f		2				1				

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
3.20	10	ZF287	3/4 10	f	f		3								
3.20	11	ZF288	3/4 11	f	f		2								
3.20	12	ZF289	3/4 12	f	f		2								
3.20	13	ZF290	3/4 13	f	f		2		1	1					
3.20	14	ZF291	3/4 14	f	f		2								
3.20	15	ZF292	3/4 15	m	m							2			
3.20	16	ZF293	3/4 16	m	m							2			
3.20	17	ZF294	3/4 17	f	f		3								
3.20	18	ZF295	3/4 18	f	f		2								
3.20	19	ZF296	3/4 19	f	f		3								
3.20	20	ZF297	3/4 20	f	f		2								
10.0	1	ZF298	4/1 1	f	f		3								
10.0	2	ZF299	4/1 2	f	f		2								
10.0	3	ZF300	4/1 3	f	f		2			1					
10.0	4	ZF301	4/1 4	f	f		3								
10.0	5	ZF302	4/1 5	f	f		2		1	1					

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
10.0	6	ZF303	4/1 6	f	f		2								
10.0	7	ZF304	4/1 7	m	m							2			
10.0	8	ZF305	4/1 8	m	m							2			
10.0	9	ZF306	4/1 9	m	m							2			
10.0	10	ZF307	4/1 10	f	f		3		1	2					
10.0	11	ZF308	4/1 11	f	f		2								
10.0	12	ZF309	4/1 12	m	m							2			
10.0	13	ZF310	4/1 13	m	m							2			
10.0	14	ZF311	4/1 14	m	m							2			
10.0	15	ZF312	4/1 15	f	f		3	1		1					
10.0	16	ZF313	4/1 16	m	m							2			
10.0	17	ZF314	4/1 17	m	m							1			
10.0	18	ZF315	4/1 18	m	m							2			
10.0	19	ZF316	4/1 19	m	m							2			
10.0	1	ZF317	4/2 1	f	f		2								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
10.0	2	ZF318	4/2 2	m	m	hermap hrodite	1					2	1		
10.0	3	ZF319	4/2 3	f	f		3								
10.0	4	ZF320	4/2 4	f	f		2								
10.0	5	ZF321	4/2 5	f	f		3			1					
10.0	6	ZF322	4/2 6	f	f		2								
10.0	7	ZF323	4/2 7	m	m							2			
10.0	8	ZF324	4/2 8	m	m							2			
10.0	9	ZF325	4/2 9	m	m							2			
10.0	10	ZF326	4/2 10	m	m							2			
10.0	11	ZF327	4/2 11	m	m							2			
10.0	12	ZF328	4/2 12	m	m							3			
10.0	13	ZF329	4/2 13	f	f		3								
10.0	14	ZF330	4/2 14	f	f		2	1							
10.0	15	ZF331	4/2 15	m	m							2			
10.0	16	ZF332	4/2 16	m	m							2			

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
10.0	17	ZF333	4/2 17	f	f		2								
10.0	18	ZF334	4/2 18	m	m							2			
10.0	19	ZF335	4/2 19	m	m							2			
10.0	20	ZF336	4/2 20	m	m							2			
10.0	1	ZF337	4/3 1	m	m							2			
10.0	2	ZF338	4/3 2	f	f		3								
10.0	3	ZF339	4/3 3	f	f		3	1	2	2	2				
10.0	4	ZF340	4/3 4	f	f		2								
10.0	5	ZF341	4/3 5	f	f		2								
10.0	6	ZF342	4/3 6	m	m							1			
10.0	7	ZF343	4/3 7	m	m							2			
10.0	8	ZF344	4/3 8	m	m							2			
10.0	9	ZF345	4/3 9	f	f		2								
10.0	10	ZF346	4/3 10	m	m							2			
10.0	11	ZF347	4/3 11	f	f		2	1		2					
10.0	12	ZF348	4/3 12	f	f		2								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
10.0	13	ZF349	4/3 13	f	f		3								
10.0	14	ZF350	4/3 14	m	m							2			
10.0	15	ZF351	4/3 15	f	f		2								
10.0	16	ZF352	4/3 16	m	m							2			
10.0	17	ZF353	4/3 17	m	m							2			
10.0	18	ZF354	4/3 18	f	f		2								
10.0	19	ZF355	4/3 19	m	m							2			
10.0	1	ZF356	4/4 1	f	f		3								
10.0	2	ZF357	4/4 2	f	f		2								
10.0	3	ZF358	4/4 3	f	f		2								
10.0	4	ZF359	4/4 4	f	f		2								
10.0	5	ZF360	4/4 5	m	m							2			
10.0	6	ZF361	4/4 6	m	m							2			
10.0	7	ZF362	4/4 7	m	m							2			
10.0	8	ZF363	4/4 8	m	m							2			
10.0	9	ZF364	4/4 9	f	f		2								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
10.0	10	ZF365	4/4 10	f	f		2								
10.0	11	ZF366	4/4 11	f	f		3								
10.0	12	ZF367	4/4 12	f	f		3								
10.0	13	ZF368	4/4 13	f	f		2								
10.0	14	ZF369	4/4 14	m	m							2			
10.0	15	ZF370	4/4 15	m	m							2			
10.0	16	ZF371	4/4 16	f	f		2								
10.0	17	ZF372	4/4 17	m	m							2			
10.0	18	ZF373	4/4 18	f	f		2								
10.0	19	ZF374	4/4 19	m	m							2			
10.0	20	ZF375	4/4 20	f	f		2								
32.0	1	ZF376	5/1 1	f	f		3	1							
32.0	2	ZF377	5/1 2	f	f		2								
32.0	3	ZF378	5/1 3	m	m							2			
32.0	4	ZF379	5/1 4	f	f		3								
32.0	5	ZF380	5/1 5	f	f		3								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
32.0	6	ZF381	5/1 6	f	f		2								
32.0	7	ZF382	5/1 7	f	f		2	1	1	1					
32.0	8	ZF383	5/1 8	f	f		3								
32.0	9	ZF384	5/1 9	f	f		2	1	3	1	2				
32.0	10	ZF385	5/1 10	f	f		2				1				
32.0	11	ZF386	5/1 11	f	f		2								
32.0	12	ZF387	5/1 12	f	f		2		1	1					
32.0	13	ZF388	5/1 13	f	f		3								
32.0	14	ZF389	5/1 14	f	f		2								
32.0	15	ZF390	5/1 15	f	f		2								
32.0	16	ZF391	5/1 16	f	f		2		1	1					
32.0	17	ZF392	5/1 17	f	f		2	1	2	1	1				
32.0	18	ZF393	5/1 18	m	m							2			
32.0	19	ZF394	5/1 19	f	f		3								
32.0	20	ZF395	5/1 20	m	m							2			
32.0	1	ZF396	5/2 1	f	f		2								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
32.0	2	ZF397	5/2 2	f	f		2	1	1		1				
32.0	3	ZF398	5/2 3	f	f		2		1		1				
32.0	4	ZF399	5/2 4	f	f		2			1					
32.0	5	ZF400	5/2 5	f	f		2			1					
32.0	6	ZF401	5/2 6	f	f		3		2	1					
32.0	7	ZF402	5/2 7	f	f		3	1			1				
32.0	8	ZF403	5/2 8	f	f		2			1					
32.0	9	ZF404	5/2 9	f	f		3	1	1						
32.0	10	ZF405	5/2 10	f	f		2			1					
32.0	11	ZF406	5/2 11	m	m							2			
32.0	12	ZF407	5/2 12	m	m							2			
32.0	13	ZF408	5/2 13	m	m							2			
32.0	14	ZF409	5/2 14	m	m							2			
32.0	15	ZF410	5/2 15	f	f		2				1				
32.0	16	ZF411	5/2 16	m	m							1			
32.0	17	ZF412	5/2 17	f	f		3								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
32.0	18	ZF413	5/2 18	f	f		2								
32.0	19	ZF414	5/2 19	m	m							2			
32.0	1	ZF415	5/3 1	f	f		2								
32.0	2	ZF416	5/3 2	m	m							2			
32.0	3	ZF417	5/3 3	f	f		3								
32.0	4	ZF418	5/3 4	f	f		3								
32.0	5	ZF419	5/3 5	f	f		3								
32.0	6	ZF420	5/3 6	f	f		2								
32.0	7	ZF421	5/3 7	m	m							1			
32.0	8	ZF422	5/3 8	m	m							2			
32.0	9	ZF423	5/3 9	m	m							2			
32.0	10	ZF424	5/3 10	m	m	hermap hrodite	1					2			
32.0	11	ZF425	5/3 11	m	m							2			
32.0	12	ZF426	5/3 12	f	f		2								
32.0	13	ZF427	5/3 13	f	f		3								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
32.0	14	ZF428	5/3 14	f	f		2								
32.0	15	ZF429	5/3 15	f	f		3								
32.0	16	ZF430	5/3 16	m	m							2			
32.0	17	ZF431	5/3 17	m	m							2			
32.0	18	ZF432	5/3 18	m	m							2			
32.0	19	ZF433	5/3 19	m	m							2			
32.0	20	ZF434	5/3 20	f	f		2								
32.0	1	ZF435	5/4 1	f	f		2								
32.0	2	ZF436	5/4 2	f	f		3								
32.0	3	ZF437	5/4 3	f	f		2								
32.0	4	ZF438	5/4 4	f	f		3								
32.0	5	ZF439	5/4 5	m	m							2			
32.0	6	ZF440	5/4 6	m	m							2			
32.0	7	ZF441	5/4 7	f	f		3								
32.0	8	ZF442	5/4 8	f	f		2								
32.0	9	ZF443	5/4 9	m	m							2			

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
32.0	10	ZF444	5/4 10	f	f		2								
32.0	11	ZF445	5/4 11	m	m							2			
32.0	12	ZF446	5/4 12	f	f		3								
32.0	13	ZF447	5/4 13	f	f		2								
32.0	14	ZF448	5/4 14	f	f		3								
32.0	15	ZF449	5/4 15	f	f		2		1						
32.0	16	ZF450	5/4 16	f	f		2								
32.0	17	ZF451	5/4 17	f	f		2								
32.0	18	ZF452	5/4 18	f	f		2		1	1					
32.0	19	ZF453	5/4 19	f	f		3								
32.0	20	ZF454	5/4 20	f	f		2		1	1					
100	1	ZF455	6/1 1	f	f		3								
100	2	ZF456	6/1 2	m	m							2			
100	3	ZF457	6/1 3	m	m							2			
100	4	ZF458	6/1 4	m	m							2			
100	5	ZF459	6/1 5	m	m							1			

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
100	6	ZF460	6/1 6	m	m							2			
100	7	ZF461	6/1 7	f	f		3								
100	8	ZF462	6/1 8	f	f		2	1		1					
100	9	ZF463	6/1 9	f	f		2	1	1						
100	10	ZF464	6/1 10	m	m							2			
100	11	ZF465	6/1 11	m	m							2			
100	12	ZF466	6/1 12	m	m							2			
100	13	ZF467	6/1 13	m	m							2			
100	14	ZF468	6/1 14	f	f		3								
100	15	ZF469	6/1 15	f	f		2								
100	16	ZF470	6/1 16	f	f		2								
100	17	ZF471	6/1 17	m	m							2			
100	18	ZF472	6/1 18	f	f		2								
100	1	ZF473	6/2 1	f	f		2		1						
100	2	ZF474	6/2 2	f	f		3		2	1					
100	3	ZF475	6/2 3	f	f		3		3		1				

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
100	4	ZF476	6/2 4	f	f		3			1					
100	5	ZF477	6/2 5	f	f		3	1	1	2					
100	6	ZF478	6/2 6	f	f		2								
100	7	ZF479	6/2 7	f	f		2								
100	8	ZF480	6/2 8	f	f		2			1					
100	9	ZF481	6/2 9	f	f		2			1					
100	10	ZF482	6/2 10	f	f		3		2	1					
100	11	ZF483	6/2 11	f	f		3								
100	12	ZF484	6/2 12	f	f		2								
100	13	ZF485	6/2 13	f	f		2		1	1					
100	14	ZF486	6/2 14	f	f		3								
100	15	ZF487	6/2 15	f	f		3		3						
100	16	ZF488	6/2 16	f	f		2		1		2				
100	17	ZF489	6/2 17	m	m							2			
100	18	ZF490	6/2 18	m	m							2			
100	19	ZF491	6/2 19	f	f		2								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
100	1	ZF492	6/3 1	f	f		3		1	1	1				
100	2	ZF493	6/3 2	m	m							2			
100	3	ZF494	6/3 3	m	m							2			
100	4	ZF495	6/3 4	f	f		2								
100	5	ZF496	6/3 5	f	f		3								
100	6	ZF497	6/3 6	f	f		3		2	1					
100	7	ZF498	6/3 7	f	f		2								
100	8	ZF499	6/3 8	m	m							2			
100	9	ZF500	6/3 9	m	m							2			
100	10	ZF501	6/3 10	f	f		3								
100	11	ZF502	6/3 11	m	m							2			
100	12	ZF503	6/3 12	f	f		2			1					
100	13	ZF504	6/3 13	f	f		2								
100	14	ZF505	6/3 14	f	f		2								
100	15	ZF506	6/3 15	m	m							2			
100	16	ZF507	6/3 16	f	f		2								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
100	17	ZF508	6/3 17	f	f		2								
100	18	ZF509	6/3 18	f	f		3								
100	19	ZF510	6/3 19	f	f		2								
100	20	ZF511	6/3 20	f	f		2								
100	1	ZF512	6/4 1	m	m							2			
100	2	ZF513	6/4 2	f	f		2								
100	3	ZF514	6/4 3	m	m							2			
100	4	ZF515	6/4 4	m	m							2			
100	5	ZF516	6/4 5	f	f		3								
100	6	ZF517	6/4 6	f	f		2								
100	7	ZF518	6/4 7	f	f		3								
100	8	ZF519	6/4 8	f	f		2								
100	9	ZF520	6/4 9	f	f		2								
100	10	ZF521	6/4 10	f	f		3								
100	11	ZF522	6/4 11	f	f		2								
100	12	ZF523	6/4 12	f	f		3								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
100	13	ZF524	6/4 13	f	f		3								
100	14	ZF525	6/4 14	m	m							2			
100	15	ZF526	6/4 15	f	f		2								
100	16	ZF527	6/4 16	f	f		3								
100	17	ZF528	6/4 17	f	f		2								
100	18	ZF529	6/4 18	m	m							2			
100	19	ZF530	6/4 19	f	f		2								
100	20	ZF531	6/4 20	f	f		3								
100	19	ZF491	6/2 19	f	f		2								
100	1	ZF492	6/3 1	f	f		3		1	1	1				
100	2	ZF493	6/3 2	m	m							2			
100	3	ZF494	6/3 3	m	m							2			
100	4	ZF495	6/3 4	f	f		2								
100	5	ZF496	6/3 5	f	f		3								
100	6	ZF497	6/3 6	f	f		3		2	1					
100	7	ZF498	6/3 7	f	f		2								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
100	8	ZF499	6/3 8	m	m							2			
100	9	ZF500	6/3 9	m	m							2			
100	10	ZF501	6/3 10	f	f		3								
100	11	ZF502	6/3 11	m	m							2			
100	12	ZF503	6/3 12	f	f		2			1					
100	13	ZF504	6/3 13	f	f		2								
100	14	ZF505	6/3 14	f	f		2								
100	15	ZF506	6/3 15	m	m							2			
100	16	ZF507	6/3 16	f	f		2								
100	17	ZF508	6/3 17	f	f		2								
100	18	ZF509	6/3 18	f	f		3								
100	19	ZF510	6/3 19	f	f		2								
100	20	ZF511	6/3 20	f	f		2								
100	1	ZF512	6/4 1	m	m							2			
100	2	ZF513	6/4 2	f	f		2								
100	3	ZF514	6/4 3	m	m							2			

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
100	4	ZF515	6/4 4	m	m							2			
100	5	ZF516	6/4 5	f	f		3								
100	6	ZF517	6/4 6	f	f		2								
100	7	ZF518	6/4 7	f	f		3								
100	8	ZF519	6/4 8	f	f		2								
100	9	ZF520	6/4 9	f	f		2								
100	10	ZF521	6/4 10	f	f		3								
100	11	ZF522	6/4 11	f	f		2								
100	12	ZF523	6/4 12	f	f		3								
100	13	ZF524	6/4 13	f	f		3								
100	14	ZF525	6/4 14	m	m							2			
100	15	ZF526	6/4 15	f	f		2								
100	16	ZF527	6/4 16	f	f		3								
100	17	ZF528	6/4 17	f	f		2								
100	18	ZF529	6/4 18	m	m							2			
100	19	ZF530	6/4 19	f	f		2								

Nominal concentration Dexamethasone [µg/L]	Fish-No.	Histo-No.	treatment	Sex macro	Sex histo	comment	ovary stage	increased oocyte atresia	egg debris	granulomatous inflammation	increased post-ovulatory follicles	testis stage	testis-ova	increased proportion of spermatogonia	granulomatous inflammation
100	20	ZF531	6/4 20	f	f		3								

Table 211: F_1 generation, maturation stage, males; control, 0.32, 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.33, 0.91 and 3.19 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.14)

	Nominal concentration Dexamethasone [µg/L]															
	Control				**0.32**				**1.00**				**3.20**			
	Mean measured concentration Dexamethasone [µg/L]															
	Control				**0.33**				**0.91**				**3.19**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Maturation stage, males**															
1	2	-(*)	-	-	-	-	-	2	-	-	-	2	-	-	2	-
2	2		-	-	-	-	-	-	-	-	-	-	-	2	2	-
3	-		-	-	-	-	-	-	-	-	3	-	-	-	-	-
4	2		2	-	-	-	-	-	-	2	-	-	-	2	-	2
5	-		-	2	-	-	2	-	-	-	2	-	-	-	3	-
6	-		-	2	2	-	1	-	-	-	2	-	-	-	2	-
7	2		-	2	-	2	2	-	-	-	2	-	-	-	2	-
8	2		2	-	-	-	2	-	-	-	2	-	-	-	2	2
9	2		2	-	2	2	-	-	-	-	-	2	-	-	2	-
10	-		2	-	3	2	-	2	-	-	-	2	-	2	1	-
11	-		-	-	2	2	-	-	-	-	-	2	2	2	-	-
12	-		2	-	2	-	-	-	2	2	-	-	-	2	-	-
13	-		2	2	-	-	-	-	2	2	-	-	2	2	-	-
14	-		2	2	2	2	2	-	-	2	2	-	2	2	-	-
15	2		2	-	-	-	2	-	-	-	2	-	-	-	2	2
16	2		-	-	-	-	-	-	-	-	2	-	2	-	2	2
17	-		-	-	-	-	-	2	-	-	-	-	-	-	2	-
18	-		-	2	2	-	2	-	-	-	2	2	-	2	2	-
19	2		-	2	3	2	-	2	2	-	-	2	-	-	2	-
20	2		2	-	2	2	-	2	-	-	-	2	-	2	-	-
Median	**2.0**	**-**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

Table 212: F_1 generation, maturation stage, males; 10.0, 32.0 and 100 µg Dexamethasone/L (nominal), respective 10.5, 34.7 and 100 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.14)

	Nominal concentration Dexamethasone [µg/L]											
	10.0				32.0				100			
	Mean measured concentration Dexamethasone [µg/L]											
	10.5				34.7				100			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Maturation stage, males**											
1	-	-	2	-	-	-	-	-	-	-	-	2
2	-	2(*)	-	-	-	-	-	-	2	-	2	-
3	-	-	-	-	2	-	-	-	2	-	2	2
4	-	-	-	-	-	-	-	-	2	-	-	2
5	-	-	-	2	-	-	-	2	1	-	-	-
6	-	-	1	2	-	-	-	2	2	-	-	-
7	2	2	2	2	-	-	1	-	-	-	-	-
8	2	2	2	2	-	-	2	-	-	-	2	-
9	2	2	-	-	-	-	2	2	-	-	2	-
10	-	2	2	-	-	-	2(*)	-	2	-	-	-
11	-	2	-	-	-	2	2	2	2	-	2	-
12	2	3	-	-	-	2	-	-	2	-	-	-
13	2	-	-	-	-	2	-	-	2	-	-	-
14	2	-	2	2	-	2	-	-	-	-	-	2
15	-	2	-	2	-	-	-	-	-	-	2	-
16	2	2	2	-	-	1	2	-	-	-	-	-
17	1	-	2	2	-	-	2	-	2	2	-	-
18	2	2	-	-	2	-	2	-	-	2	-	2
19	2	2	2	2	-	2	2	-	-	-	-	-
20	-	2	-	-	2	-	-	-	-	-	-	-
Median	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**

(*) Both fishes were determined as hermaphrodite and thus not included in the calculation of the median.

Table 213: F_1 generation, maturation stage, females; control, 0.32, 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.33, 0.91 and 3.19 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.14)

	Nominal concentration Dexamethasone [µg/L]															
	control				0.32				1.00				3.20			
	Mean measured concentration Dexamethasone [µg/L]															
	Control				0.33				0.91				3.19			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Maturation stage, females**															
1	-	-(*)	2	2	3	3	2	-	3	3	3	-	2	2	-	2
2	-		2	2	2	2	2	2	2	2	2	2	2	-	-	2
3	2		2	2	2	3	2	2	2	2	-	2	3	2	2	2
4	-		-	3	3	2	3	2	3	-	2	2	2	-	2	-
5	2		2	-	2	3	-	2	2	3	-	2	3	3	-	2
6	2		2	-	-	2	-	2	2	2	-	2	2	2	-	3
7	-		2	-	2	-	-	3	3	2	-	2	3	3	-	2
8	-		-	3	2	2	-	2	2	2	-	2	2	2	-	-
9	-		-	2	-	-	2	2	2	3	3	-	2	2	-	2
10	3		-	3	-	-	3	-	2	3	2	-	3	-	-	3
11	3		2	2	-	-	2	2	2	2	2	-	-	-	3	2
12	2		-	2	-	3	2	2	-	-	2	2	2	-	2	2
13	2		-	-	2	3	2	2	-	-	2	3	-	-	2	2
14	2		-	-	-	-	-	2	2	-	-	2	-	-	2	2
15	-		-	2	3	2	-	2	3	2	-	2	2	2	-	-
16	-		3	2	2	3	2	2	2	3	-	2	-	3	-	-
17	3		2	2	2	3	2	-	1	2	2	3	3	2	-	3
18	3		3	-	-	3	-	2	2	2	-	-	2	-	-	2
19	-		2	-	-	-	2	-	-	2	1	-	3	2	-	3
20	-		-	2	-	-	2	-	2	2	-	-	-	-	-	2
Median	**2.0**	**-**	**2.0**	**2.0**	**2.0**	**3.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**	**2.0**

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

Table 214: F_1 generation, maturation stage, females; 10.0, 32.0 and 100 µg Dexamethasone/L (nominal), respective 10.5, 34.7 and 100 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.14)

	Nominal concentration Dexamethasone [µg/L]											
	10.0				32.0				100			
	Mean measured concentration Dexamethasone [µg/L]											
	10.5				34.7				100			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Maturation stage, females**											
1	3	2	-	3	3	2	2	2	3	2	3	-
2	2	1(*)	3	2	2	2	-	3	-	3	-	2
3	2	3	3	2	-	2	3	2	-	3	-	-
4	3	2	2	2	3	2	3	3	-	3	2	-
5	2	3	2	-	3	2	3	-	-	3	3	3
6	2	2	-	-	2	3	2	-	-	2	3	2
7	-	-	-	-	2	3	-	3	3	2	2	3
8	-	-	-	-	3	2	-	2	2	2	-	2
9	-	-	2	2	2	3	-	-	2	2	-	2
10	3	-	-	2	2	2	1(*)	2	-	3	3	3
11	2	-	2	3	2	-	-	-	-	3	-	2
12	-	-	2	3	2	-	2	3	-	2	2	3
13	-	3	3	2	3	-	3	2	-	2	2	3
14	-	2	-	-	2	-	2	3	3	3	2	-
15	3	-	2	-	2	2	3	2	2	3	-	2
16	-	-	-	2	2	-	-	2	2	2	2	3
17	-	2	-	-	2	3	-	2	-	-	2	2
18	-	-	2	2	-	2	-	2	2	-	3	-
19	-	-	-	-	3	-	-	3	-	2	2	2
20	-	-	-	2	-	-	2	2	-	-	2	3
Median	**2**	**2**	**2**	**2**	**2**	**2**	**3**	**2**	**2**	**2**	**2**	**2**

(*) Both fishes were determined as hermaphrodite and thus not included in the calculation of the median.

Table 215: F$_1$ generation, maturation stage, summary

		Nominal concentration Dexamethasone [µg/L]						
		control	0.32	1.00	3.20	10.0	32.0	100
		Mean measured concentration Dexamethasone [µg/L]						
	Replicate	control	0.33	0.91	3.19	10.5	34.4	100
Maturation stage males, median value	A	2	2	2	2	2	2	2
	B	-	2	2	2	2	2	2
	C	2	2	2	2	2	2	2
	D	2	2	2	2	2	2	2
Maturation stage females, median value	A	2	2	2	2	2	2	2
	B	-	3	2	2	2	2	2
	C	2	2	2	2	2	3	2
	D	2	2	2	2	2	2	2

Table 216: F_1, increased oocyte atresia, females; control, 0.32, 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.33, 0.91 and 3.19 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.14)

	Nominal concentration Dexamethasone [µg/L]															
	control				0.32				1.00				3.20			
	Mean measured concentration Dexamethasone [µg/L]															
	Control				0.33				0.91				3.19			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Increased oocyte atresia, females															
1	-	- (*)	0	0	0	0	0	-	0	0	0	-	0	0	-	0
2	-		1	0	0	0	0	0	0	0	0	0	0	-	-	0
3	0		0	0	0	0	0	0	0	1	-	0	0	0	0	0
4	-		-	0	0	0	0	1	0	-	0	0	0	-	0	-
5	0		0	-	1	0	-	0	0	0	-	0	0	0	-	0
6	0		0	-	-	0	-	0	0	0	-	0	0	0	-	0
7	-		0	-	0	-	-	0	0	0	-	0	0	0	-	0
8	-		-	0	0	0	-	0	0	0	-	0	0	0	-	-
9	-		-	1	-	-	1	0	0	0	0	-	0	0	-	0
10	0		-	0	-	-	0	-	0	0	0	-	0	-	-	0
11	0		1	0	-	-	0	0	0	0	0	-	-	-	0	0
12	0		-	0	-	0	0	0	-	-	1	0	0	-	0	0
13	0		-	-	0	0	0	1	-	-	0	0	-	-	1	0
14	0		-	-	-	-	-	0	0	-	-	0	-	-	0	0
15	-		-	0	2	0	-	0	0	0	-	0	0	0	-	-
16	-		0	0	0	0	0	0	0	0	-	0	-	0	-	-
17	0		0	0	0	1	0	-	0	0	0	0	0	0	-	0
18	0		0	-	-	0	-	1	0	0	-	-	0	-	-	0
19	-		0	-	-	-	0	-	-	0	0	-	1	0	-	0
20	-		-	0	-	-	1	-	2	0	-	-	-	-	-	0
Median	**0.0**	**-**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

Table 217: F_1, increased oocyte atresia, females; 10.0, 32.0 and 100 µg Dexamethasone/L (nominal), respective 10.5, 34.7 and 100 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.14)

	Nominal concentration Dexamethasone [µg/L]											
	10.0				**32.0**				**100**			
	Mean measured concentration Dexamethasone [µg/L]											
	10.5				**34.7**				**100**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Increased oocyte atresia, females**											
1	0	0	-	0	1	0	0	0	0	0	0	-
2	0	h(*)	0	0	0	1	-	0	-	0	-	0
3	0	0	1	0	-	0	0	0	-	0	-	-
4	0	0	0	0	0	0	0	0	-	0	0	-
5	0	0	0	-	0	0	0	-	-	1	0	0
6	0	0	-	-	0	0	0	-	-	0	0	0
7	-	-	-	-	1	1	-	0	0	0	0	0
8	-	-	-	-	0	0	-	0	1	0	-	0
9	-	-	0	0	1	1	-	-	1	0	-	0
10	0	-	-	0	0	0	h(*)	0	-	0	0	0
11	0	-	1	0	0	-	-	-	-	0	-	0
12	-	-	0	0	0	-	0	0	-	0	0	0
13	-	0	0	0	0	-	0	0	-	0	0	0
14	-	1	-	-	0	-	0	0	0	0	0	-
15	1	-	0	-	0	0	0	0	0	0	-	0
16	-	-	-	0	0	-	-	0	0	0	0	0
17	-	0	-	-	1	0	-	0	-	-	0	0
18	-	-	0	0	-	0	-	0	0	-	0	-
19	-	-	-	-	0	-	-	0	-	0	0	0
20	-	-	-	0	-	-	0	0	-	-	0	0
Median	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

(*) Both fishes were determined as hermaphrodite (h) and thus excluded from any further calculation.

Table 218: F_1, egg debris, females; control, 0.32, 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.33, 0.91 and 3.19 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.14)

	Nominal concentration Dexamethasone [µg/L]															
	control				**0.32**				**1.00**				**3.20**			
	Mean measured concentration Dexamethasone [µg/L]															
	Control				**0.33**				**0.91**				**3.19**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Egg debris, females**															
1	-	-(*)	1	0	0	0	1	-	0	0	1	-	0	0	-	0
2	-		2	0	0	0	1	0	0	0	0	0	0	-	-	0
3	0		0	0	0	0	0	0	0	2	-	0	0	0	0	0
4	-		-	0	0	0	0	0	0	-	0	0	0	-	0	-
5	0		0	-	1	0	-	0	0	0	-	0	0	0	-	0
6	0		0	-	-	0	-	0	0	0	-	0	1	0	-	0
7	-		0	-	0	-	-	0	1	0	-	0	0	1	-	0
8	-		-	0	0	0	-	0	0	0	-	0	0	0	-	-
9	-		-	1	-	-	1	0	0	0	0	-	0	0	-	0
10	0		-	0	-	-	1	-	0	0	0	-	0	-	-	0
11	0		0	0	-	-	0	0	0	0	0	-	-	-	0	0
12	0		-	0	-	0	0	0	-	-	0	0	1	-	0	0
13	1		-	-	0	0	0	0	-	-	0	0	-	-	0	1
14	0		-	-	-	-	-	0	0	-	-	0	-	-	2	0
15	-		-	1	0	0	-	1	0	0	-	0	0	0	-	-
16	-		0	0	0	0	0	0	0	0	-	0	-	0	-	-
17	0		0	0	0	1	0	-	0	1	0	0	0	0	-	0
18	1		0	-	-	0	-	0	0	2	-	-	0	-	-	0
19	-		0	-	-	-	0	-	-	0	0	-	1	1	-	0
20	-		-	1	-	-	1	-	1	0	-	-	-	-	-	0
Median	**0.0**	**-**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

Table 219: F_1, egg debris, females; 10.0, 32.0 and 100 µg Dexamethasone/L (nominal) respective 10.5, 34.7 and 100 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.14)

	Nominal concentration Dexamethasone [µg/L]											
	10.0				**32.0**				**100**			
	Mean measured concentration Dexamethasone [µg/L]											
	10.5				**34.7**				**100**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Egg debris, females**											
1	0	0	-	0	0	0	0	0	0	1	1	-
2	0	h(*)	0	0	0	1	-	0	-	2	-	0
3	0	0	2	0	-	1	0	0	-	3	-	-
4	0	0	0	0	0	0	0	0	-	0	0	-
5	1	0	0	-	0	0	0	-	-	1	0	0
6	0	0	-	-	0	2	0	-	-	0	2	0
7	-	-	-	-	1	0	-	0	0	0	0	0
8	-	-	-	-	0	0	-	0	0	0	-	0
9	-	-	0	0	3	1	-	-	1	0	-	0
10	1	-	-	0	0	0	h(*)	0	-	2	0	0
11	0	-	0	0	0	-	-	-	-	0	-	0
12	-	-	0	0	1	-	0	0	-	0	0	0
13	-	0	0	0	0	-	0	0	-	1	0	0
14	-	0	-	-	0	-	0	0	0	0	0	-
15	0	-	0	-	0	0	0	1	0	3	-	0
16	-	-	-	0	1	-	-	0	0	1	0	0
17	-	0	-	-	2	0	-	0	-	-	0	0
18	-	-	0	0	-	0	-	1	0	-	0	-
19	-	-	-	-	0	-	-	0	-	0	0	0
20	-	-	-	0	-	-	0	1	-	-	0	0
Median	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

(*) Both fishes were determined as hermaphrodite (h) and thus not included in the calculation of the median.

Table 220: F_1, granulomatous inflammation, females; control, 0.32, 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.33, 0.91 and 3.19 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.14)

	Nominal concentration Dexamethasone [µg/L]															
	control				0.32				1.00				3.20			
	Mean measured concentration Dexamethasone [µg/L]															
	Control				0.33				0.91				3.19			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Granulomatous inflammation, females**															
1	-	- (*)	0	0	1	1	1	-	0	0	1	-	2	0	-	0
2	-		0	0	0	0	0	0	0	0	0	0	0	-	-	0
3	0		0	0	0	0	1	0	0	1	-	0	0	0	0	0
4	-		-	0	0	0	0	0	0	-	0	0	0	-	1	-
5	0		0	-	1	0	-	0	0	0	-	1	0	0	-	0
6	0		0	-	-	0	-	0	0	0	-	0	0	0	-	0
7	-		0	-	1	-	-	0	0	1	-	0	0	0	-	0
8	-		-	0	0	0	-	0	0	0	-	0	1	0	-	-
9	-		-	2	-	-	0	0	0	0	0	-	1	0	-	0
10	0		-	0	-	-	0	-	0	0	0	-	0	-	-	0
11	0		0	0	-	-	0	0	1	0	0	-	-	-	0	0
12	0		-	0	-	0	1	0	-	-	0	0	0	-	0	0
13	0		-	-	0	0	1	0	-	-	0	0	-	-	0	1
14	0		-	-	-	-	-	0	1	-	-	0	-	-	1	0
15	-		-	0	0	0	-	0	0	0	-	0	0	0	-	-
16	-		0	0	0	0	0	0	0	0	-	0	-	3	-	-
17	0		0	1	0	0	0	-	1	0	0	0	0	0	-	0
18	0		1	-	-	0	-	0	0	0	-	-	0	-	-	0
19	-		0	-	-	-	0	-	-	0	0	-	0	0	-	0
20	-		-	0	-	-	1	-	1	0	-	-	-	-	-	0
Median	**0.0**	**-**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

Table 221: F_1, granulomatous inflammation, females; 10.0, 32.0 and 100 µg Dexamethasone/L (nominal), respective 10.5, 34.7 and 100 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.14)

	Nominal concentration Dexamethasone [µg/L]											
	10.0				**32.0**				**100**			
	Mean measured concentration Dexamethasone [µg/L]											
	10.5				**34.7**				**100**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Granulomatous inflammation, females**											
1	0	0	-	0	0	0	0	0	0	0	1	-
2	0	h(*)	0	0	0	0	-	0	-	1	-	0
3	1	0	2	0	-	0	0	0	-	0	-	-
4	0	0	0	0	0	1	0	0	-	1	0	-
5	1	1	0	-	0	1	0	-	-	2	0	0
6	0	0	-	-	0	1	0	-	-	0	1	0
7	-	-	-	-	1	0	-	0	0	0	0	0
8	-	-	-	-	0	1	-	0	1	1	-	0
9	-	-	0	0	1	0	-	-	0	1	-	0
10	2	-	-	0	0	1	h(*)	0	-	1	0	0
11	0	-	2	0	0	-	-	-	-	0	-	0
12	-	-	0	0	1	-	0	0	-	0	1	0
13	-	0	0	0	0	-	0	0	-	1	0	0
14	-	0	-	-	0	-	0	0	0	0	0	-
15	1	-	0	-	0	0	0	0	0	0	-	0
16	-	-	-	0	1	-	-	0	0	0	0	0
17	-	0	-	-	1	0	-	0	-	-	0	0
18	-	-	0	0	-	0	-	1	0	-	0	-
19	-	-	-	-	0	-	-	0	-	0	0	0
20	-	-	-	0	-	-	0	1	-	-	0	0
Median	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

(*) Both fishes were determined as hermaphrodite (h) and thus not included in the calculation of the median.

Table 222: F_1, increased post-ovulatory follicles, females; control, 0.32, 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.33, 0.91 and 3.19 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.14)

	Nominal concentration Dexamethasone [µg/L]															
	Control				0.32				1.00				3.20			
	Mean measured concentration Dexamethasone [µg/L]															
	Control				0.33				0.91				3.19			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Increased post-ovulatory follicles, females															
1	-	-(*)	0	0	0	0	0	-	0	0	0	-	0	0	-	0
2	-		0	0	0	0	0	1	0	0	0	0	0	-	-	0
3	0		1	0	0	0	0	0	1	2	-	0	0	0	0	0
4	-		-	0	0	0	0	1	0	-	0	0	0	-	0	-
5	0		1	-	0	0	-	0	0	0	-	0	0	0	-	0
6	0		1	-	-	0	-	0	0	0	-	0	1	0	-	0
7	-		0	-	0	-	-	0	0	0	-	0	0	0	-	0
8	-		-	0	1	0	-	0	1	1	-	0	1	0	-	-
9	-		-	0	-	-	0	0	0	0	0	-	0	0	-	1
10	0		-	0	-	-	0	-	0	0	0	-	0	-	-	0
11	0		0	0	-	-	0	0	2	0	0	-	-	-	0	0
12	0		-	0	-	0	0	0	-	-	0	0	0	-	0	0
13	0		-	-	0	0	1	0	-	-	0	0	-	-	0	0
14	0		-	-	-	-	-	0	0	-	-	0	-	-	3	0
15	-		-	0	0	0	-	0	0	0	-	0	0	0	-	-
16	-		0	0	1	0	1	1	0	0	-	0	-	0	-	-
17	0		0	0	0	0	0	-	0	0	0	0	0	0	-	0
18	0		0	-	-	0	-	1	0	1	-	-	0	-	-	0
19	-		0	-	-	-	0	-	-	0	0	-	1	3	-	0
20	-		-	0	-	-	0	-	2	0	-	-	-	-	-	0
Median	**0.0**	**-**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

Table 223: F_1, increased post-ovulatory follicles, females; 10.0, 32.0 and 100 µg Dexamethasone/L (nominal), respective 10.5, 34.7 and 100 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.14)

	Nominal concentration Dexamethasone [µg/L]											
	10.0				**32.0**				**100**			
	Mean measured concentration Dexamethasone [µg/L]											
	10.5				**34.7**				**100**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Increased post-ovulatory follicles, females**											
1	0	0	-	0	0	0	0	0	0	0	1	-
2	0	h(*)	0	0	0	1	-	0	-	0	-	0
3	0	0	2	0	-	1	0	0	-	1	-	-
4	0	0	0	0	0	0	0	0	-	0	0	-
5	0	0	0	-	0	0	0	-	-	0	0	0
6	0	0	-	-	0	0	0	-	-	0	0	0
7	-	-	-	-	0	1	-	0	0	0	0	0
8	-	-	-	-	0	0	-	0	0	0		0
9	-	-	0	0	2	0	-	-	0	0		0
10	0	-	-	0	1	0	h(*)	0	-	0	0	0
11	0	-	0	0	0	-	-	-	-	0		0
12	-	-	0	0	0	-	0	0	-	0	0	0
13	-	0	0	0	0	-	0	0	-	0	0	0
14	-	0	-	-	0	-	0	0	0	0	0	-
15	0	-	0	-	0	1	0	0	0	0		0
16	-	-	-	0	0	-	-	0	0	2	0	0
17	-	0	-	-	1	0	-	0	-		0	0
18	-	-	0	0	-	0	-	0	0		0	-
19	-	-	-	-	0	-	-	0	-	0	0	0
20	-	-	-	0	-	-	0	0	-	-	0	0
Median	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

(*) Both fishes were determined as hermaphrodite (h) and thus not included in the calculation of the median.

Table 224: F_1, testis-ova, males; control, 0.32, 1.00 and 3.20 µg Dexamethasone/L (nominal), respective 0.33, 0.91 and 3.19 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.14)

	Nominal concentration Dexamethasone [µg/L]															
	Control				0.32				1.00				3.20			
	Mean measured concentration Dexamethasone [µg/L]															
	Control				0.33				0.91				3.19			
Replicate	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Fish no.	Testis-ova, males															
1	0	-(*)	-	-	-	-	-	0	-	-	-	0	-	-	0	-
2	0		-	-	-	-	-	-	-	-	-	-	-	0	0	-
3	-		-	-	-	-	-	-	-	-	0	-	-	-	-	-
4	0		0	-	-	-	-	-	-	0	-	-	-	0	-	0
5	-		-	0	-	-	0	-	-	-	0	-	-	-	0	-
6	-		-	0	0	-	0	-	-	-	0	-	-	-	0	-
7	0		-	0	-	0	0	-	-	-	0	-	-	-	0	-
8	0		0	-	-	-	0	-	-	-	0	-	-	-	0	0
9	0		0	-	0	0	-	-	-	-	-	0	-	-	0	-
10	-		0	-	0	0	-	0	-	-	-	0	-	0	0	-
11	-		-	-	0	0	-	-	-	-	-	0	0	0	-	-
12	-		0	-	0	-	-	-	0	0	-	-	-	0	-	-
13	-		0	1	-	-	-	-	0	0	-	-	0	0	-	-
14	-		0	0	0	0	0	-	-	0	0	-	0	0	-	-
15	0		0	-	-	-	0	-	-	-	0	-	-	-	0	0
16	0		-	-	-	-	-	-	-	-	0	-	0	-	0	0
17	-		-	-	-	-	-	0	-	-	-	-	-	-	0	-
18	-		-	0	0	-	0	-	-	-	0	0	-	0	0	-
19	0		-	0	0	0	-	0	0	-	-	0	-	-	0	-
20	0		0	-	0	0	-	0	-	-	-	0	-	0	-	-
Median	**0.0**	**-**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

Table 225: F_1, testis-ova, males; 10.0, 32.0 and 100 µg Dexamethasone/L (nominal), respective 10.5, 34.7 and 100 µg Dexamethasone/L (mean measured); (based on histological data provided in C.2.14)

	Nominal concentration Dexamethasone [µg/L]											
	10.0				**32.0**				**100**			
	Mean measured concentration Dexamethasone [µg/L]											
	10.5				**34.7**				**100**			
Replicate	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
Fish no.	**Testis-ova, males**											
1	-	-	0	-	-	-	-	-	-	-	-	0
2	-	h(*)	-	-	-	-	0	-	0	-	0	-
3	-	-	-	-	0	-	-	-	0	-	0	0
4	-	-	-	-	-	-	-	-	0	-	-	0
5	-	-	-	0	-	-	-	0	0	-	-	-
6	-	-	0	0	-	-	-	0	0	-	-	-
7	0	0	0	0	-	-	0	-	-	-	-	-
8	0	0	0	0	-	-	0	-	-	-	0	-
9	0	0	-	-	-	-	0	0	-	-	0	-
10	-	0	0	-	-	-	h(*)	-	0	-	-	-
11	-	0	-	-	-	0	0	0	0	-	0	-
12	0	0	-	-	-	0	-	-	0	-	-	-
13	0	-	-	-	-	0	-	-	0	-	-	-
14	0	-	0	0	-	0	-	-	-	-	-	0
15	-	0	-	0	-	-	-	-	-	-	0	-
16	0	0	0	-	-	0	0	-	-	-	-	-
17	0	-	0	0	-	-	0	-	0	0	-	-
18	0	0	-	-	0	-	0	-	-	0	-	0
19	0	0	0	0	-	0	0	-	-	-	-	-
20	-	0	-	-	0	-	-	-	-	-	-	-
Median	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

(*) Both fishes were determined as hermaphrodite (h) and thus not included in the calculation of the median.

Table 226: F1 generation, gonadal lesions severity, summary

		Nominal concentration Dexamethasone [µg/L]						
		control	0.32	1.00	3.20	10.0	32.0	100
		Mean measured concentration Dexamethasone [µg/L]						
	Replicate	control	0.33	0.91	3.19	10.5	34.7	100
Testis-ova, males, median value	A	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	B	-(*)	0.0	0.0	0.0	0.0	0.0	0.0
	C	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Increased oocyte atresia, females, median value	A	0.0	0.0	0.0	0.0	0.0	0.0	0
	B	-(*)	0.0	0.0	0.0	0.0	0.0	0
	C	0.0	0.0	0.0	0.0	0.0	0.0	0
	D	0.0	0.0	0.0	0.0	0.0	0.0	0
Egg debris, females, median value	A	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	B	-(*)	0.0	0.0	0.0	0.0	0.0	0.0
	C	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Granumatomous inflammation, females, median value	A	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	B	-(*)	0.0	0.0	0.0	0.0	0.0	0.0
	C	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Increased post-ovulatory follicles, females, median value	A	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	B	-(*)	0.0	0.0	0.0	0.0	0.0	0.0
	C	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0

(*) Larvae of replicate B got lost due to a technical failure at an age of around 20 days. It was decided not to restart this group.

C.2.15 Statistics, treatment with Dexamethasone, parental generation (F_0)

All biological endpoints were statistically evaluated with ToxRat (ToxRat® Professional 3.3.0; ToxRat® Solutions GmbH, Alsdorf, Germany). Only statistically significant results are presented in the following. Please note, that the nominal concentrations are given.

► **Fertilisation rate, parental generation (F_0)**

Data were arcsine transformed prior evaluation.

Table 227: Fertilisation rate of *Danio rerio* as dependent on concentration of the test item and time (F_0)

Treatm. [µg/L]	Control	0.3	1.0	3.2	10.0	32.0	100.0
	1.263	1.153	1.192	1.179	1.154	1.139	1.256
	1.328	1.223	1.124	1.233	1.217	1.171	1.254
	1.217	1.255	1.213	1.214	1.038	1.208	1.243
	1.272	1.194	1.247	1.231	1.150	1.196	1.268
Mean	**1.270**	**1.206**	**1.194**	**1.214**	**1.140**	**1.179**	**1.255**
Std.Dev.	0.0453	0.0432	0.0520	0.0252	0.0744	0.0308	0.0102
n	4	4	4	4	4	4	4
CV	3.6	3.6	4.4	2.1	6.5	2.6	0.8

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from Tabs F0 Fertility (arcsine transformed)).

The normality check was passed ($p > 0.01$). The Levene test indicated variance homogeneity ($p > 0.010$). Thus, the variance homogeneity check was passed. As normal-distribution and variance-homogeneity requirements were fulfilled, a parametric multiple test was advisable.

The analysis of contrasts did not reveal a linear trend; thus, the selected Williams test was replaced by Dunnett`s test.

Table 228: Dunnett´s multiple t-test procedure (fertility, F_0)

Treatm. [ng/L]	Mean	s	df	%MDD	t	t*	Sign
Control	1.270	0.04447					
0.3	1.206	0.04447	21	-6.1	-2.03	-2.45	-
1.0	1.194	0.04447	21	-6.1	-2.43	-2.45	-
3.2	1.214	0.04447	21	-6.1	-1.78	-2.45	-
10.0	**1.140**	**0.04447**	**21**	**-6.1**	**-4.14**	**-2.45**	**+**
32.0	**1.179**	**0.04447**	**21**	**-6.1**	**-2.91**	**-2.45**	**+**
100.0	1.255	0.04447	21	-6.1	-0.46	-2.45	-

+: significant; -: non-significant; Comparison of treatments with "Control". Significance was Alpha = 0.050. one-sided smaller (multiple level); Mean: arithmetic mean; n: sample size; s: standard deviation; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; t*: critical t for Ho: μ1 = μ2 = ... = μk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments).

► Fecundity (egg number per day and female), parental generation (F_0)

Table 229: Fecundity [n] of *Danio rerio* (Zebrafish) as dependent on concentration of the test item and time (F_0)

Treatm. [µg/L]	Control	0.3	1.0	3.2	10.0	32.0	100.0
	82.7	63.7	39.1	65.7	82.3	82.5	80.8
	70.0	53.3	50.6	52.2	65.5	67.5	66.4
	64.6	50.0	64.7	59.8	75.3	67.2	61.8
	80.9	60.9	55.6	68.7	67.7	53.3	57.8
Mean	**74.5**	**57.0**	**52.5**	**61.6**	**72.7**	**67.6**	**66.7**
Std.Dev.	8.68	6.41	10.67	7.26	7.67	11.95	10.06
n	4	4	4	4	4	4	4
CV	11.6	11.3	20.3	11.8	10.6	17.7	15.1

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from Tabs F0 Fecundity).

The normality check was passed (p > 0.01). The Levene test indicated variance homogeneity (p > 0.010). Thus, the variance homogeneity check was passed. As normal-distribution and variance-homogeneity requirements were fulfilled, a parametric multiple test was advisable.

The analysis of contrasts did not reveal a linear trend; thus, the selected Williams test was replaced by Dunnett`s test.

Table 230: Dunnett´s multiple t-test procedure (fecundity, F_0)

Treatm. [ng/L]	Mean	s	df	%MDD	t	t*	Sign
Control	74.5	9.148					
0.3	**57.0**	**9.148**	**21**	**-21.3**	**-2.72**	**-2.45**	**+**
1.0	**52.5**	**9.148**	**21**	**-21.3**	**-3.40**	**-2.45**	**+**
3.2	61.6	9.148	21	-21.3	-2.00	-2.45	-
10.0	72.7	9.148	21	-21.3	-0.28	-2.45	-
32.0	67.6	9.148	21	-21.3	-1.07	-2.45	-
100.0	66.7	9.148	21	-21.3	-1.21	-2.45	-

+: significant; -: non-significant; Comparison of treatments with "Control". Significance was Alpha = 0.050. one-sided smaller (multiple level); Mean: arithmetic mean; n: sample size; s: standard deviation; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; t*: critical t for Ho: µ1 = µ2 = ... = µk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments).

► Total length [cm] females at test end, parental generation (F_0)

Table 231: Total length females of *Danio rerio* as dependent on concentration of the test item and time (F_0, at test end)

Treatm. [µg/L]	Control	0.3	1.0	3.2	10.0	32.0	100.0
	4.0	4.0	3.9	3.8	3.9	3.9	3.7
	4.0	4.2	3.9	3.9	3.7	3.8	3.9
	3.9	3.9	3.7	4.0	3.9	3.7	3.7
	3.9	3.6	3.9	3.7	4.0	4.0	3.7
Mean	**3.9**	**3.9**	**3.8**	**3.8**	**3.9**	**3.8**	**3.8**
Std.Dev.	0.06	0.23	0.10	0.11	0.12	0.12	0.09
n	4	4	4	4	4	4	4
CV	1.4	5.9	2.6	2.9	3.2	3.3	2.4

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from Tabs F_0 Total length females at test end).

The normality check was passed ($p > 0.01$). The Levene test indicated variance homogeneity ($p > 0.010$). Thus, the variance homogeneity check was passed. As normal-distribution and variance-homogeneity requirements were fulfilled, a parametric multiple test was advisable.

The analysis of contrasts revealed a linear trend; thus, the Williams test was performed.

Table 232: Williams Multiple Sequential t-test Procedure (total length females at test end, F_0)

Treatm. [ng/L]	Mean	s	df	LhM	%MDD	t	t*	Sign
Control	3.9	0.130						
0.3	3.9	0.130	21	3.9	-4.0	-0.38	-1.72	-
1.0	3.8	0.130	21	3.8	-4.2	-1.03	-1.80	-
3.2	3.8	0.130	21	3.9	-4.3	-0.86	-1.83	-
10.0	3.9	0.130	21	3.9	-4.3	-0.86	-1.84	-
32.0	3.8	0.130	21	3.8	-4.3	-1.10	-1.85	-
100.0	**3.8**	**0.130**	**21**	**3.8**	**-4.3**	**-2.01**	**-1.86**	**+**

+: significant; -: non-significant

Comparison of treatments with "Control" by the t test procedure after Williams with F0 total length females at test end: Significance was Alpha = 0.050. one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: $\mu1 = \mu2 = ... = \mu k$; the differences are significant in case $|t| > |t^*|$ (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered.

Based on this, a NOEC of 32.0 µg Dexamethasone/L was determined. This is corresponding to the mean measured concentration of 34.7 µg Dexamethasone/L.

► Wet weight males at test end, parental generation (F_0)

Table 233: Wet weight males of *Danio rerio* as dependent on concentration of the test item and time (F_0, test end)

Treatm. [µg/L]	Control	0.3	1.0	3.2	10.0	32.0	100.0
	0.474	0.475	0.461	0.465	0.416	0.422	0.463
	0.434	0.494	0.485	0.506	0.508	0.441	0.375
	0.507	0.513	0.458	0.508	0.650	0.434	0.390
	0.512	0.445	0.455	0.465	0.443	0.425	0.395
Mean	**0.482**	**0.482**	**0.465**	**0.486**	**0.504**	**0.430**	**0.406**
Std.Dev.	0.0358	0.0286	0.0138	0.0239	0.1043	0.0087	0.0389
n	4	4	4	4	4	4	4
CV	7.4	5.9	3.0	4.9	20.7	2.0	9.6

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from Tabs F_0 wet weight males at test end)

Normality check failed (p <= 0.01). The Levene test indicated variance homogeneity (p > 0.010). Thus, the variance homogeneity check was passed. As normal distribution was poore, but variance homogeneity requirements were fulfilled, a parametric multiple test was performed.

The analysis of contrasts revealed a linear trend, thus the SD Jonckheere-Terpstra test was performed.

Table 234: Step-down Jonckheere-Terpstra Test Procedure (wet weight males at test end, F_0)

Treatm. [ng/L]	Mean	Med	n	J	J*	p(J)	Sign.
Control	0.482	0.490	4				
0.3	0.482	0.485	4	2.0		0.443	-
1.0	0.465	0.460	4	10.0	0.73	0.232	-
3.2	0.486	0.485	4	0.0	0.00	0.500	-
10.0	0.504	0.476	4	2.0	0.07	0.527	-
32.0	**0.430**	**0.429**	**4**	**72.0**	**1.82**	**0.035**	**+**
100.0	**0.406**	**0.392**	**4**	**146.0**	**2.92**	**0.002**	**+**

+: significant; -: non-significant

Step-down Jonckheere-Terpstra test procedure with F_0 wet weight males at test end: Step-down test to detect a trend in decreasing median effects on f0 wet weight males (Alpha is 0.050; one-sided smaller); Med: median. n: sample size; J: test statistic; J*: standardized J (in case a value for J* is shown for a treatment. the large-sample approximation was calculated (= sum of all replicates N > 11)); p(J): probability that the observed trend could be due to chance; Ho is accepted. if p(J) > Alpha. Note that the step-down test terminates after the first non-significant treatment is encountered.

Based on this, a NOEC of 10.0 µg Dexamethasone/L was determined. This is corresponding to the mean measured concentration of 10.5 µg Dexamethasone/L.

► Wet weight females at test end, parental generation (F_0)

Table 235: Wet weight females of *Danio rerio* as dependent on concentration of the test item and time (F_0, test end)

Treatm. [µg/L]	Control	0.3	1.0	3.2	10.0	32.0	100.0
	0.736	0.740	0.731	0.694	0.636	0.669	0.545
	0.761	0.812	0.711	0.683	0.610	0.614	0.614
	0.671	0.693	0.613	0.714	0.650	0.567	0.544
	0.646	0.544	0.702	0.608	0.713	0.703	0.534
Mean	**0.704**	**0.697**	**0.689**	**0.675**	**0.652**	**0.638**	**0.559**
Std.Dev.	0.0543	0.1134	0.0520	0.0462	0.0438	0.0598	0.0369
n	4	4	4	4	4	4	4
CV	16.3	7.5	6.9	6.7	9.4	6.6	16.3

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from Tabs F_0 wet weight females at test end).

The normality check was passed (p > 0.01). The Levene test indicated variance homogeneity (p > 0.010). Thus, the variance homogeneity check was passed. As normal-distribution and variance-homogeneity requirements were fulfilled, a parametric multiple test was advisable.

The analysis of contrasts revealed a linear trend; thus, the Williams test was performed.

Table 236: Williams Multiple Sequential t-test Procedure (wet weight females at test end, F_0)

Treatm. [ng/L]	Mean	s	df	LhM	%MDD	t	t*	Sign
Control	0.704	0.06268						
0.3	0.697	0.06268	21	0.697	-10.8	-0.14	-1.72	-
1.0	0.689	0.06268	21	0.689	-11.4	-0.32	-1.80	-
3.2	0.675	0.06268	21	0.675	-11.5	-0.65	-1.83	-
10.0	0.652	0.06268	21	0.652	-11.6	-1.16	-1.84	-
32.0	0.638	0.06268	21	0.638	-11.7	-1.47	-1.85	-
100.0	**0.559**	**0.06268**	**21**	**0.559**	**-11.7**	**-3.26**	**-1.86**	**+**

Comparison of treatments with "Control" by the t test procedure after Williams with F_0 wet weight females at test end: Significance was Alpha = 0.050. one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: µ1 = µ2 = ... = µk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered.

Based on this, a NOEC of 32.0 µg Dexamethasone/L was determined. This is corresponding to the mean measured concentration of 34.7 µg Dexamethasone/L.

C.2.16 Statistics, treatment with Dexamethasone, F_1 generation

All endpoints were statistically evaluated with ToxRat (ToxRat® Professional 3.3.0; ToxRat® Solutions GmbH, Alsdorf, Germany). Only statistically significant results are presented in the following. Please note, that the nominal concentrations are given.

► **Total length [cm] at day 35 pf, first filial generation (F_1)**

Table 237: Total length [cm] of *Danio rerio* as dependent on concentration of the test item and time (F_1, day 35 pf)

Treatm. [µg/L]	Control	0.3	1.0	3.2	10.0	32.0	100.0
	1.8	1.8	1.8	1.7	1.8	1.8	1.6
	-	1.8	1.7	1.8	1.8	1.7	1.7
	1.8	1.8	1.7	1.7	2.0	1.7	1.5
	1.8	1.9	1.8	1.8	1.8	1.7	1.6
Mean	**1.8**	**1.8**	**1.8**	**1.7**	**1.8**	**1.7**	**1.6**
Std.Dev.	0.01	0.06	0.04	0.04	0.12	0.06	0.07
n	3	4	4	4	4	4	4
CV	0.4	3.6	2.5	2.6	6.6	3.3	4.2

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from Tabs $F_{1.}$ total length at day 35 pf)

Normality check failed (p <= 0.01). The Levene test indicated variance homogeneity (p > 0.010). Thus, the variance homogeneity check was passed. As normal distribution was poore, but variance homogeneity requirements were fulfilled, a parametric multiple test was performed.

The analysis of contrasts revealed a linear trend, thus the SD Jonckheere-Terpstra test was performed.

Table 238. Step-down Jonckheere-Terpstra Test Procedure (total length at day 35 pf, F_1)

Treatm. [ng/L]	Mean	Med	n	J	J*	p(J)	Sign.
Control	1.8	1.8	3				
0.3	1.8	1.8	4	4.0		0.314	-
1.0	1.8	1.8	4	2.0		0.468	-
3.2	1.7	1.7	4	23.0	1.19	0.118	-
10.0	1.8	1.8	4	3.0	0.11	0.543	-
32.0	1.7	1.7	4	29.0	0.78	0.218	-
100.0	**1.6**	**1.6**	**4**	**117.0**	**2.47**	**0.007**	**+**

+: significant; -: non-significant

Step-down Jonckheere-Terpstra test procedure with f1. total length at day 35 pf: Step-down test to detect a trend in decreasing median effects on f1. total length. 35dpf (Alpha is 0.050; one-sided smaller); Med: median. n: sample size; J: test statistic; J*: standardized J (in case a value for J* is shown for a treatment. the large-sample approximation was calculated (= sum of all replicates N > 11)); p(J): probability that the observed trend could be due to chance; Ho is accepted. if p(J) > Alpha. Note that the step-down test terminates after the first non-significant treatment is encountered

Based on this, a NOEC of 32.0 µg Dexamethasone/L was determined. This is corresponding to the mean measured concentration of 34.7 µg Dexamethasone/L.

► Total length at day 63 pf, first filial generation (F_1)

Table 239: Total length [cm] of *Danio rerio* as dependent on concentration of the test item and time (F_1, day 63 pf)

Treatm. [µg/L]	Control	0.3	1.0	3.2	10.0	32.0	100.0
	3.1	3.1	3.1	2.9	3.0	3.0	2.7
	-	3.1	3.1	3.1	2.9	2.9	2.8
	3.0	3.0	3.1	3.0	3.1	2.9	2.7
	3.0	3.0	3.0	3.0	3.0	2.8	2.7
Mean	**3.0**	**3.0**	**3.1**	**3.0**	**3.0**	**2.9**	**2.7**
Std.Dev.	0.02	0.06	0.04	0.09	0.09	0.07	0.05
n	3	4	4	4	4	4	4
CV	0.6	2.0	1.2	2.9	3.0	2.6	2.0

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from Tabs F_1. Total length, day 63 pf)

The normality check was passed (p > 0.01). The Levene test indicated variance homogeneity (p > 0.010). Thus, the variance homogeneity check was passed. As normal-distribution and variance-homogeneity requirements were fulfilled, a parametric multiple test was advisable.

The analysis of contrasts revealed a linear trend; thus, the Williams test was performed.

Table 240: Williams Multiple Sequential t-test Procedure (total length at day 63 pf, F_1)

Treatm. [ng/L]	Mean	s	df	LhM	%MDD	t	t*	Sign
Control	3.0	0.066						
0.3	3.0	0.066	20	3.1	-2.9	0.19	-1.73	-
1.0	3.1	0.066	20	3.1	-3.0	0.19	-1.82	-
3.2	3.0	0.066	20	3.0	-3.1	-0.63	-1.85	-
10.0	3.0	0.066	20	3.0	-3.1	-0.63	-1.86	-
32.0	**2.9**	**0.066**	**20**	**2.9**	**-3.1**	**-2.68**	**-1.87**	**+**
100.0	**2.7**	**0.066**	**20**	**2.7**	**-3.1**	**-6.05**	**-1.88**	**+**

+: significant; -: non-significant

Comparison of treatments with "Control" by the t test procedure after Williams with f1. total length at 63dpf: Significance was Alpha = 0.050. one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: µ1 = µ2 = ... = µk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered.

Based on this result, a NOEC of 10.0 µg Dexamethasone/L was determined. This is corresponding to the mean measured concentration of 10.5 µg Dexamethasone/L.

► Total length males at test end, first filial generation (F_1)

Table 241: Total length males [cm] of *Danio rerio* as dependent on concentration of the test item and time (F_1, test end)

Treatm. [µg/L]	Control	0.3	1.0	3.2	10.0	32.0	100.0
	3.9	3.9	3.8	3.9	3.9	3.7	3.4
	-	4.0	3.6	3.9	3.7	3.7	3.5
	3.7	3.7	3.7	3.9	3.9	3.5	3.5
	3.6	3.9	3.9	3.8	3.7	3.5	3.4
Mean	**3.8**	**3.9**	**3.8**	**3.9**	**3.8**	**3.6**	**3.5**
Std.Dev.	0.12	0.09	0.10	0.05	0.09	0.11	0.06
n	3	4	4	4	4	4	4
CV	3.2	2.4	2.7	1.3	2.4	3.1	1.6

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from Tabs F_1, total length males, test end).

The normality check was passed (p > 0.01). The Levene test indicated variance homogeneity (p > 0.010). Thus, the variance homogeneity check was passed. As normal-distribution and variance-homogeneity requirements were fulfilled, a parametric multiple test was advisable.

The analysis of contrasts revealed a linear trend; thus, the Williams test was performed.

Table 242: Williams Multiple Sequential t-test Procedure (total length males at test end, F_1)

Treatm. [ng/L]	Mean	s	df	LhM	%MDD	t	t*	Sign
Control	3.8	0.091						
0.3	3.9	0.091	20	3.9	-3.2	1.58	-1.73	-
1.0	3.8	0.091	20	3.8	-3.4	0.75	-1.82	-
3.2	3.9	0.091	20	3.8	-3.4	0.75	-1.85	-
10.0	3.8	0.091	20	3.8	-3.4	0.74	-1.86	-
32.0	**3.6**	**0.091**	**20**	**3.6**	**-3.5**	**-2.55**	**-1.87**	**+**
100.0	**3.5**	**0.091**	**20**	**3.5**	**-3.5**	**-4.13**	**-1.88**	**+**

+: significant; -: non-significant; Comparison of treatments with "Control" by the t test procedure after Williams with f1 total length males at Test end: Significance was Alpha = 0.050. one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: $\mu1 = \mu2 = ... = \mu k$; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered.

Based on this, a NOEC of 10.0 µg Dexamethasone/L was determined. This is corresponding to the mean measured concentration of 10.5 µg Dexamethasone/L.

► Wet weight males at test end, first filial generation (F_1)

Table 243: Wet weight males [g] of *Danio rerio* as dependent on concentration of the test item and time (F_1, test end)

Treatm. [µg/L]	Control	0.3	1.0	3.2	10.0	32.0	100.0
	0.444	0.446	0.414	0.463	0.442	0.356	0.296
	-	0.511	0.401	0.487	0.414	0.366	0.296
	0.416	0.421	0.443	0.449	0.532	0.366	0.298
	0.414	0.438	0.468	0.414	0.393	0.348	0.293
Mean	**0.425**	**0.454**	**0.432**	**0.453**	**0.445**	**0.359**	**0.296**
Std.Dev.	0.0170	0.0395	0.0301	0.0306	0.0610	0.0088	0.0021
n	3	4	4	4	4	4	4
CV	4.0	8.7	7.0	6.7	13.7	2.5	0.7

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from Tabs F_1 wet weight males at test end)

The normality check was passed (p > 0.01). The Levene test indicated variance homogeneity (p > 0.010). Thus, the variance homogeneity check was passed. As normal-distribution and variance-homogeneity requirements were fulfilled, a parametric multiple test was advisable.

The analysis of contrasts revealed a linear trend; thus, the Williams test was performed.

Table 244. Williams Multiple Sequential t-test Procedure (wet weight males at test end, F_1)

Treatm. [ng/L]	Mean	s	df	LhM	%MDD	t	t*	Sign
Control	0.425	0.03332						
0.3	0.454	0.03332	20	0.454	-10.3	1.15	-1.73	-
1.0	0.432	0.03332	20	0.443	-10.9	0.73	-1.82	-
3.2	0.453	0.03332	20	0.443	-11.1	0.73	-1.85	-
10.0	0.445	0.03332	20	0.443	-11.2	0.73	-1.86	-
32.0	**0.359**	**0.03332**	**20**	**0.359**	**-11.2**	**-2.58**	**-1.87**	**+**
100.0	**0.296**	**0.03332**	**20**	**0.296**	**-11.3**	**-5.07**	**-1.88**	**+**

+: significant; -: non-significant

Comparison of treatments with "Control" by the t test procedure after Williams with f1 wet weight males at Test end: Significance was Alpha = 0.050. one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: $\mu1 = \mu2 = ... = \mu k$; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered.

Based on this, a NOEC of 10.0 µg Dexamethasone/L was determined. This is corresponding to the mean measured concentration of 10.5 µg Dexamethasone/L.

► Wet weight females at test end, first filial generation (F_1)

Table 245: Wet weight females [g] of *Danio rerio* as dependent on concentration of the test item and time (F_1, test end)

Treatm. [µg/L]	Control	0.3	1.0	3.2	10.0	32.0	100.0
	0.509	0.616	0.572	0.533	0.525	0.469	0.398
	-	0.616	0.617	0.603	0.528	0.522	0.445
	0.559	0.531	0.536	0.460	0.532	0.435	0.388
	0.517	0.529	0.546	0.543	0.537	0.409	0.344
Mean	**0.528**	**0.573**	**0.568**	**0.534**	**0.531**	**0.459**	**0.394**
Std.Dev.	0.0266	0.0500	0.0363	0.0587	0.0052	0.0488	0.0415
n	3	4	4	4	4	4	4
CV	5.0	8.7	6.4	11.0	1.0	10.6	10.5

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from Tabs F_1 wet weight females at test end).

The normality check was passed (p > 0.01). The Levene test indicated variance homogeneity (p > 0.010). Thus, the variance homogeneity check was passed. As normal-distribution and variance-homogeneity requirements were fulfilled, a parametric multiple test was advisable.

The analysis of contrasts revealed a linear trend; thus, the Williams test was performed.

Table 246: Williams Multiple Sequential t-test Procedure (wet weight females at test end, F_1)

Treatm. [ng/L]	Mean	s	df	LhM	%MDD	t	t*	Sign
Control	0.528	0.04220						
0.3	0.573	0.04220	20	0.573	-10.5	1.39	-1.73	-
1.0	0.568	0.04220	20	0.568	-11.1	1.22	-1.82	-
3.2	0.534	0.04220	20	0.534	-11.3	0.19	-1.85	-
10.0	0.531	0.04220	20	0.531	-11.4	0.07	-1.86	-
32.0	**0.459**	**0.04220**	**20**	**0.459**	**-11.4**	**-2.16**	**-1.87**	**+**
100.0	**0.394**	**0.04220**	**20**	**0.394**	**-11.5**	**-4.18**	**-1.88**	**+**

+: significant; -: non-significant
Comparison of treatments with "Control" by the t test procedure after Williams with F_1 wet weight females at test end: Significance was Alpha = 0.050. one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; LhM: max. likelihood mean; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; 't*: critical t for Ho: µ1 = µ2 = ... = µk; the differences are significant in case |t| > |t*| (The residual variance of an ANOVA was applied; df = N - k; N: sum of treatment replicates n(i); k: number of treatments). Note that the step-down test terminates after the first non-significant treatment is encountered.

Based on this, a NOEC of 10.0 µg Dexamethasone/L was determined. This is corresponding to the mean measured concentration of 10.5 µg Dexamethasone/L.

C.2.17 Statistics, treatment with Dexamethasone, F_2 generation

Please note, that the nominal concentrations are given.

► Hatching rate [%], data arcsine transformed prior to evaluation

Table 247: F_2 hatching rate [%] of *Danio rerio* as dependent on concentration of the test item and time (data arcsine transformed prior to evaluation)

Treatm. [µg/L]	Control	0.3	1.0	3.2	10.0	32.0	100.0
	1.571	1.571	1.571	1.345	1.345	1.173	1.345
	-	1.571	1.571	1.571	1.107	1.345	1.571
	1.571	1.571	1.571	1.345	1.345	1.173	1.345
	1.571	1.571	1.571	1.571	1.249	1.345	1.571
Mean	**1.571**	**1.571**	**1.571**	**1.458**	**1.262**	**1.259**	**1.458**
Std.Dev.	0.0000	0.0000	0.0000	0.1302	0.1126	0.0994	0.1302
n	3	4	4	4	4	4	4
CV	0.0	0.0	0.0	8.9	8.9	7.9	8.9

Mean: arithmetic mean; Std.Dev.: standard deviation; n: number of replicates; CV: coefficient of variation (from Tabs F2 Hatching success%).

The normality check was passed ($p > 0.01$). The Levene test indicates variance heterogeneity ($p <= 0.010$).

However. normal distribution requirements were fulfilled. The Welch-t-test for non-homogeneous variances with Bonferroni-Holm-adjustment was advisable.

Table 248: Multiple Sequentially-rejective Welsh-t-test After Bonferroni-Holm (hatching rate, F_2)

Treatm. [ng/L]	Mean	s	df	%MDD	t	p(t)	Alpha(i)	Sign
Control	1.571	0.0000						
0.3	1.571	0.0000	n.d.	n.d.	n.d.	n.d.	n.d.	*
1.0	1.571	0.0000	n.d.	n.d.	n.d.	n.d.	n.d.	*
3.2	1.458	0.1302	4	-8.8	-1.73	0.079	0.050	-
10.0	**1.262**	**0.1126**	**4**	**-11.4**	**-5.49**	**0.003**	**0.017**	**+**
32.0	**1.259**	**0.0994**	**4**	**-11.1**	**-6.27**	**0.002**	**0.013**	**+**
100.0	1.458	0.1302	4	-11.5	-1.73	0.079	0.025	-

+: significant; -: non-significant; *: test could not be performed; n.d.: not determined.

Multiple sequentially rejective comparisons of treatments with "Control". Significance was Alpha = 0.050. one-sided smaller; Mean: arithmetic mean; n: sample size; s: standard deviation; MDD: minimum detectable difference to Control (in percent of Control); t: sample t; p(t): probability of sample t for Ho: $\mu1 = \mu2$; Alpha(i): adjusted significance levels; the differences are significant in case p(t) <= Alpha(i); dfm: modified degrees of freedom due to heteroscedasticity.(Control(c) and treatment(t) variance was applied: $s^2(c)/nc + s^2(t)/nt$. each). Note that the step-down test terminates after the first non-significant treatment is encountered.

D Appendix: Literature Research and References

D.1 Results of the literature research for progestins

Table 249: Results of the literature research for progestins

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
1	adult zebrafish	Megestrol acetate (MTA)	33-666 ng/L	21 d exposure	> Egg production was decreased at 666 ng/L > The exposure significantly decreased the circulating concentrations of estradiol in female fish or 11-keto testosterone (11-KT) in male fish(E2) and testosterone (T); downregulated the transcription of certain genes along the hypothalamic-pituitary-gonadal (HPG) axis > MTA did not affect early embryonic development or hatching success in the F1 generation	The synthetic progestin megestrol acetate adversely affects zebrafish reproduction	Han et al., Aquatic Toxicology 150 (2014) 66–72
2	adult fathead minnow and yeast	Levonorgestrel, Gestodene, Desogestrel, Drospirenone	1 ng/L - 10 µg/L	21 d exposure of fish 1st experiment: exposure to 100 ng/L 2nd experiment: exposure to Gestodene at 1, 10, and 100 ng/L and Desogestrel at	> Exposure to a single concentration of 100 ng/L: Levonorgestrel and Gestodene, fish stopped spawning almost completely. Desogestrel and Drospirenone did not affect reproduction. > In 2nd experiment exposure to: Gestodene concentrations as low as 1 ng/L had significant effects on reproduction over 21 d. Desogestrel at or above 1 µg/L were required to significantly reduce egg production. The synthetic progestins also masculinized the female fish in a concentration-dependent manner.	Several Synthetic Progestins with Different Potencies Adversely Affect Reproduction of Fish	Runnalls et al., Environ. Sci. Technol. 2013, 47, 2077−2084

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
				100 ng/L, 1 µg/L, and 10 µg/L	> Results from yeast-based in vitro assays demonstrated that the progestins are all strongly androgenic.		
3	adult fathead minnow	gestodene	10 and 100ng/L	8d exposure	> No treatment effects were observed on the gonadosomatic index > Males: more aggressive and less interested in courtship and mating; Males from all treatments exhibited nuptial tubercles, fatpad, and dorsal fin spot with no visual effects from exposure to GES; > Females: less female courtship behavior, displayed maletypical courtship behaviors toward the other female; strong decrease in daily egg deposition in the 10 ng/L treatment and a complete cessation of deposition by day 3 for the 100 ng/L treatment; females showed apparent dose dependent masculinization having nuptial tubercles	Exposure to the Contraceptive Progestin, Gestodene, Alters Reproductive Behavior, Arrests Egg Deposition, and Masculinizes Development in the Fathead Minnow (*Pimephales promelas*)	Frankel et al., Environ. Sci. Technol. 2013, 47, 2077−2084
4	zebrafish embryo	Progesterone, RU486 (Mifepriston), norethindrone, levonorgestrel	2, 20, and 200 ng/L	144 h post fertilization; expressional changes of ar, esr1, vtg1, hsd17ß3, and progesterone (pgr), mineralo- (mr), and glucocorticoid (gr) receptors, each at 48, 96, and 144 hpf	Significant up to 4-fold induction of pgr, ar, mr, and hsd17b3 occurred at 2 ng/L P4 and higher, while RU484 inhibited pgr expression. Norethindrone and levonorgestrel modulated some transcripts mainly above 2 ng/L.	Progestins and Antiprogestins Affect Gene Expression in Early Development in Zebrafish (*Danio rerio*) at Environmental Concentrations	Zucchi et al., Environ. Sci. Technol. 2012, 46, 5183−5192
5	zebrafish embryo, adult zebrafish	Medroxyprogester one acetate, dydrogesterone	between 4.5 and 1663 ng/L (measured)	adult fish 21 days of exposure;	> dydrogesterone and both mixtures impaired reproductive capacities (egg production) of breeding pairs and led to histological alterations of	Synthetic Progestins Medroxyprogesterone Acetate and	Zhao et al., Environ. Sci. Technol. 2015, 49, 4636−4645

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
		and binary mixtures		embyro exposure 144 hpf	ovaries and testes and increased gonadosomatic index. > Transcriptional analysis of up to 28 genes belonging to different pathways demonstrated alterations in steroid hormone receptors, steroidogenesis enzymes, and specifically, the circadian rhythm genes, in different organs of adult zebrafish and eleuthero-embryos. Alterations occurred even at environmentally relevant concentrations of 4.5−4.8 ng/L medroxyprogesterone acetate, dydrogesterone and the mixture in eleuthero-embryos and at 43−89 ng/L in adult zebrafish. > Substances tested in mixtures displayed additive effects in most but not all parameters in adults and eleuthero-embryos	Dydrogesterone and Their Binary Mixtures Adversely Affect Reproduction and Lead to Histological and Transcriptional Alterations in Zebrafish (*Danio rerio*)	
6	Japanese medaka			established a transient transactivation assay using glucocorticoid receptors (olGRs) and mineralocorticoid receptor to analyse their functional properties	> olGR2 was highly responsive to glucocorticoids, similar to the human GR, whereas the olGR1 subtype was minimally responsive. olGR2 most likely mediates glucocorticoid signaling in medaka. > Crosstalk between GRs and other steroid hormones was tested, and found that progestins could activate or inactivate olGR2-mediating transcription, depending on the presence or absence of cortisol. > The transactivation assays developed for medaka GRs provide tools to gain useful insights into corticosteroid signaling in fish and for in vitro screening of environmental substances activating GRs.	Characterization of *Oryzias latipes* glucocorticoid receptors and their unique response to progestins	Miyagawa et al., J. Appl. Toxicol. 2015; 35: 302–309
7	adult zebrafish	progesterone (P4), drospirenone (DRS)	7 to 13650 ng/L	exposure 21 days; fecundity, histology,	> Transcriptional analysis revealed significant and dose-dependent alterations of the circadian rhythm network in the brain with little effects in the gonads.	Environmental Progestins Progesterone and Drospirenone Alter the	Zhao et al., Environ. Sci. Technol. 2015, 49, 10155−10164

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
				transcriptional analysis of the circadian rhythm network	> Significant alterations of many target transcripts occurred even at environmental relevant concentrations of 7 ng/L P4 and at 99 ng/L DRS. > Fecundity was decreased at 742 (P4) and 2763 (DRS) ng/L. > Dose-dependent alterations in the circadian rhythm network were also observed in F1 eleuthero-embryos at environmentally relevant concentrations	Circadian Rhythm Network in Zebrafish (*Danio rerio*)	
8	juvenile zebrafish	levonorgestrel, progesterone	levonorgestrel 5.5, 79 and 834 ng/L; progesterone 3.7, 77 and 1122 ng/L	exposure from 20 to 80 dpf; Histology, transcript levels of anti-Müllerian hormone (amh), Cytochrome P450 (CYP11B) and CYP19a1a	Levonorgestrel exposure caused 100% males at all concentrations tested, progesterone did not affect the sex ratio. Transcript levels of the gonadal genes amh, CYP11B and CYP19a1a indicated that the masculinizing effect of levonorgestrel occurred very rapidly. In fish exposed to levonorgestrel or progesterone gonadotropin transcript concentrations were high already at 44 dpf, indicating that both progestins caused precocious puberty. Gonad histology at 50 dpf showed a well advanced sexual maturation, but only in males. Progestins can affect sexual development in fish and levonorgestrel induces a male phenotype.	Developmental exposure to progestins causes male bias andprecocious puberty in zebrafish (*Danio rerio)*	J. Svensson et al., Aquatic Toxicology 177 (2016) 316–323
9	adult zebrafish	chlormadinone acetate	6.4 and 53,745 ng/L	exposure for 21 days, rerpoduction and transcriptional effects in brains, livers, and gonads	> chlormadinone acetate induced a slight but statistically significant reduction in fecundity at 65 ng/L and 53.745 ng/L. > Differential expression for gene transcripts of steroid hormone receptors, genes related to the hypothalamicpituitary-gonadal axis, and steroidogenesis. > significant decrease of transcript levels of vitellogenin (vtg1) in ovaries and liver, and of cyp2k7 in the liver of males, as well as a significant increase of transcripts of the progesterone receptor (pgr) in testes, and cyp2k1 in the liver of females	Reproductive and transcriptional effects of the antiandrogenic progestin chlormadinone acetate in zebrafish (*Danio rerio)*	Siegenthaler et al. / Environmental Pollution 223 (2017) 346e356

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
10	zebrafish	levonorgestrel; co-exposure to flutamide and levonorgestrel;	1, 10, 33, and 100 ng/L	zebrafish eggs 143dpf, mRNA expression levels of genes involved in sex differentiation and gonadal development	> complete sex reversal and 100% males were observed in the 10, 33 and 100 ng/L treatments > Down-regulation of the mRNA expression of aromatase (e.g., cyp19a1a, cyp19a1b), the forkhead transcription factor gene L2 (foxl2) and the Fushi tarazu factor-1d (nr5a1b). > In contrast, transcription of the doublesex and mab-3-related transcription factor 1 (dmrt1) gene was up-regulated at 33 and 100ng/L > Androgen receptor (ar) mRNA expression was significantly down-regulated at 28 and 42 dpf. > Co-exposure to flutamide (an androgen antagonist) and levonorgestrel, led to a decrease in the sex inversion potency of levonorgestrel	The progestin levonorgestrel affects sex differentiation in zebrafish at environmentally relevant concentrations	J. Hua et al. / Aquatic Toxicology 166 (2015) 1–9
11	juvenile zebrafish	progesterone, norgestrel	4, 33, 63 ng/L progesterone; 4, 34, 77 ng /L norgestrel	exposure of fish 20 to 60dpf; transcriptions of genes related to sex differentiation (Amh, Dmrt1, Figa, Sox9a and Sox9b genes), sex hormone levels and transcriptional expression profiles along the hypothalamic–pituitary–gonadal (HPG)	> progesterone: increase in proportion of females as well as significant down-regulation of Amh gene and up-regulation of Figa at a concentration of 63 ng/L > norgestrel: shift in the sex ratio toward males was observed following exposure to 34 and 77 ng/L norgestrel, together with induction of Dmrt1 gene and inhibition of Figa gene > Sex hormones were detected only in the fish exposed to the highest progesterone concentration; whereas estradiol and androstenedione were detected only in the fish of the control and lowest norgestrel concentration. > the increase in females was associated with the significant up-regulation of several key genes controlling the synthesis of sex hormones (i.e., Cyp17, Cyp19a1a and Hsd3b) following exposure to 63 ng/L progesterone > significant down-regulation of Cyp11a1, Cyp17,	Long-term exposure to environmentally relevant concentrations of progesterone and norgestrel affects sex differentiation in zebrafish (*Danio rerio*)	Y.-Q. Liang et al. / Aquatic Toxicology 160 (2015) 172–179

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
				and hypothalamic–pituitary–adrenal (HPA)	Cyp19a1a and Hsd3b genes in the male-biased populations caused by 34 and 77 ng/L norgestrel.		
12	zebrafish embyro	progesterone	6, 45 and 90 ng/L	144 hpf exposure to 0, 6, 45 and 90 ng/L progesterone; daily assessment of transcriptional expression profiles of the HPG and HPA axes	> For the receptor signaling pathways, progesterone significantly induced the transcript of Pgr gene above 45 ng L 1 at 72 and 144 hpf, but inhibited its transcript above 6 ng/L at 96 and 120 hpf. > A significant up-regulation of Vtg1 mRNA was observed at 6 ng L 1 P4 or higher at 24, 96 and 144 hpf. > For the steroidogenic pathways, the transcriptional expression of Cyp11a1 and Hsd17b3 mRNAs was mediated by 6 ng/L progesterone or higher according to different exposure time points. > progesterone resulted in a significant induction of Cyp19a1a and Cyp11b mRNA expression while it caused a significant inhibition of Hsd11b2 mRNA expression above 6 ng L 1. > For the other target genes related to hypothalamic and pituitary hormones, progesterone mainly modulated the transcripts of Gnrh2, Fshb and Lhb genes at 6 ng L 1 or higher	The effects of progesterone on transcriptional expression profiles of genes associated with hypothalamic–pituitary–gonadal and hypothalamic–pituitary–adrenal axes during the early development of zebrafish (*Danio rerio)*	Y.-Q. Liang et al. / Chemosphere 128 (2015) 199–206
13	zebrafish embyro	ethinylestradiol (EE2) and norgestrel (NGT) and in combination	36 and 5513 ng/L	96 hpf exposure	> HPG axis showed no significant changes for EE2-alone group and the mixtures caused a strong induction of Cyp19a1b and Lhb genes and the NGT alone group significantly increased the transcript of Cyp11a1 gene > circadian rhythm signaling were similar to that in the HPG axis. Single EE2 and NGT exposures had no significant effects on the expression levels of circadian rhythm genes, except for Cry4 gene with significant down-regulation.	Transcriptional alterations induced by binary mixtures of ethinylestradiol and norgestrel during the early development of zebrafish (*Danio rerio*)	Y.-Q. Liang et al. / Comparative Biochemistry and Physiology, Part C 195 (2017) 60–67

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
					> most of gene transcriptions of HPG axis (e.g., Pgr, Mprα, Esr1, Esr2a, Vtg1, Ar, Cyp11b, Star, Gnrh3 and Fshb) and circadian rhythm signaling (e.g., Cry1a, Cry2a, Cry2b,Per3, Arntl1b, Arntl2, Clock1a, Cry3 and Cry4) displayed most pronounced alterations in the mixtures as compared to single EE2 and NGT exposures. > exposure to the binary mixtures of EE2 and NGT produced significantly enhanced effects in fish as compared to single chemical exposures		
14	zebrafish embryo	17β-estradiol (E2), 17α-ethinylestradiol (EE2), testosterone (T), pregnenolone (P5), progesterone (P4), dydrogesterone (DYD), medroxyprogesterone (MEP), medroxyprogesterone acetate (MPA), megestrol acetate (MGA), chlormadinone acetate (CMA), cyproterone acetate (CPA), promegestone (R5020), nestorone (NES), nomegestrol acetate (NGA), ethisterone		4dpf, effects of set of synthetic ligands of the nuclear progesterone receptor on the glial-specific expression of the zebrafish brain aromatase (cyp19a1b)	> progesterone, dydrogesterone, drospirenone and all the progesterone-derived progestins had no effect on GFP expression. > All progestins derived from 19-nortesterone induced GFP in a concentration-dependent manner with EC50 ranging from the low nM range to hundreds nM. > Progesterone had no effect on luciferase activity while NET and LNG induced luciferase activity that was blocked by ICI182,780. > Zebrafish-ERs competition assays showed that NET and LNG were unable to bind to ERs, suggesting that the effects of these compounds on cyp19a1b require metabolic activation prior to elicit estrogenic activity. > 19-nortestosterone derived progestins elicit estrogenic activity by inducing cyp19a1b expression in radial glial cells	Several synthetic progestins disrupt the glial cell specific-brain aromatase expression in developing zebra fish	J. Cano-Nicolau et al. / Toxicology and Applied Pharmacology 305 (2016) 12–21

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
		(ETH), ethynodiol diacetate (EDA), lynestrenol (LYN), norethindrone acetate (NEA), norethindrone (NET), tibolone (TIB), desogestrel (DSG), etonogestrel (ENG), gestodene (GES), levonorgestrel (LNG), norgestimate (NTE), norgestrel (NGL), drospirenone (DRO), mifepristone (RU486), finasteride (FIN), trislostane (TRI), Gestonorone (GRN), ICI 182-780 (ICI)					
15	zebrafish	ethinylestradiol (EE2), levonorgestrel (LNG) and in mixtures	EE2 concentrations from 9.77 × 10−8 µM (28.9 pg/L) to 1 × 10−4 µM (29.6 ng/L) and LNG concentrations	expression of the brain specific ER-regulated cyp19a1b gene; > in vitro transient	> EE2 treatment induced expression of luciferase activity in a concentration-dependent manner, and EC50of 1.4 × 10^{-10} M for ERα and 6.63 × 10^{-12} M for ERβ2 were calculated. LNG was poorly effective in inducing luciferase activity and no saturation of the response was observed EC50s for LNG 36.1 × 10^{-6} M for ERα and 12.6 × 10^{-6} M for ERβ2 > mixtures of EE2 and LNG: EC50 for EE2 were 1.29	Additive effects of levonorgestrel and ethinylestradiol on brain aromatase (cyp19a1b) in zebrafish specific in vitro and in vivo bioassays	N. Hinfray et al. / Toxicology and Applied Pharmacology 307 (2016) 108–114

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
			from 0.293 nM (91 ng/L) to 0.3 µM (93µg/L)	reporter gene assay in a human glial cell line (U251-MG) co-transfected with zebrafish estrogen receptors (zfERs) and the luciferase gene under the control of the zebrafish cyp19a1b gene promoter > Exposure of transgenic zebrafish expressing GFP under the control of the zebrafish cyp19a1b gene promoter (cyp19a1b-GFP) for 96 h	$\times 10^{-10}$ M and 1.19×10^{-11} M for U251-MG glial cells transfected with ERα and ERβ2 respectively. ForLNG, EC50 were 22×10^{-6} M and 8.6×10^{-6} M for U251-MG glial cells transfected with ERα and ERβ2 respectively. EC50s are in the same range of order than those in the single test compound experiments > in vivo effects:exposure to EE2 for 96 h led to a concentration-dependent induction of GFP expression, with an EC50 of $5.0 \times 10^{-12} \pm 1.2 \times 10^{-12}$M; exposure to LNG alone led to a concentration-dependent induction of GFP expression in radial glial cells of the brain with an EC50 of 1.68×10^{-8}M $\pm 0.6 \times 10^{-8}$ M > in vivo: In mixture experiment with transgenic cyp19a1b-GFP zebrafish, EC50 were 6.18×10^{-12} M for EE2 and 4.55×10^{-8} M for LNG.		
16	wild anchovy, *Engraulis encrasicolus*			presence and effects of xenobiotics on wild anchovy Engraulis encrasicolus in theWestern Adriatic Sea	> Twenty-one PCBs and five organochlorines were detected on the order of ng/g (organochlorine pesticides, a-HCH, b-HCH, g-HCH, HCB, heptachlor, dichlorodiphenyltrichloroethane (DDT) and its metabolites (OC2 subgroup) were evaluated; > vitellogenin, vitellogenin receptor and genes encoding for the zona radiata proteins were evaluated in gonad and/or liver and found	Detection of endocrine disrupting chemicals and evidence of their effects on the HPG axis of the European anchovy Engraulis encrasicolus	A. Miccoli et 138 al. / Marine Environmental Research 127 (2017) 137e147

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
					transcribed in male specimens; in addition, intersex was histologically identified in the 13% of testis		
17	pubertal roach (*Rutilus rutilus*)	levonorgestrel (LNG)	3, 31, 312, and 3124 ng/l	exposed for 28 days	> Both males and females treated with 3124 ng/l LNG exhibited the upregulated levels of vitellogenin andoestrogen receptor 1 mRNA in the liver. At the same concentration, LNG caused a significant upregulationof the mRNA expression of the gene encoding luteinising hormone -subunit (lhβ) and the suppression of the mRNA expression of the gene encoding follicle-stimulating hormone β-subunit (fshβ) in the pituitary of both male and female roach. > Females treated with 3124 ng/l LNG exhibited significantly lower plasma11-ketotestosterone (11-KT) and oestradiol (E2) concentrations, whereas their testosterone (T) level washigher compared with the control. > A lower LNG concentration (312 ng/l) suppressed mRNA expression of fsh in males only. Females exposed to 312 ng/l LNG presented significantly lower plasma E2 concentrations. > Males exposed to ≥31 ng/l LNG exhibited significantly reduced 11-KT levels, > the ovaries of females were not affected by LNG exposure, where as the testes of males exposed to 31 and 312 ng/l LNG exhibited a significantly higher percentage of spermatogonia compared with the control. > the highest tested concentration of LNG (3124 ng/l) exerted an oestrogenic effect on fish of both sexes	The progestin levonorgestrel disrupts gonadotropin expression andsex steroid levels in pubertal roach (Rutilus rutilus)	H.K. Kroupova et al. / Aquatic Toxicology 154 (2014) 154–162

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
18	larval *Xenopus laevis*	levonorgestrel (LNG), progesterone (P4)	10 and 100 nM	Tadpoles of X. laevis at the developmental stage NF 58; gonadal-kidney complex was removed for organ culture and exposed to 10 and 100 nM for 24 h	> higher susceptibility of female over male gonads to gestagenic ED. > Only in female gonads LNG, but not P4, had direct inhibitory effects on gene expression of steroidogenic acute regulatory protein and P450 side chain cleavage enzyme, whereas aromatase expression decreased in reaction to both gestagens. > LNG drastically disrupted the thyroid system, which resembles direct effects on thyroid glands and pituitary along the pituitary-thyroid axis disturbing metamorphic developmenInent amphibians, environmental gestagens not only affect the reproductive system but at least LNG can impact also development by disruption of the thyroid system	Endocrine disruption by environmental gestagens in amphibians - A short review supported by new in vitro data using gonads of Xenopus laevis	A. Zikova et al. / Chemosphere 181 (2017) 74e82
19	Eastern Mosquitofish (*Gambusia holbrooki*)	levonorgestrel, mifepristone, 17 - trenbolone and in mixtures	0.3 g/L levonorgestrel, 0.05 g/L mifepristone, and a mixture of levonorgestrel (0.3 g/L) and mifepristone (0.05 g/L)	48h exposure; 21d exposure hepatic microarray analysis	> Microarray analysis revealed that mifepristone does not act as an anti-progestagen in G. holbrooki in liver tissues, and that levonorgestrel elicits strong effects on the processes of embryo development and lipid transport. > Levonorgestrel induced male secondary sexual characteristic formation in females, and co-exposure of either an androgenor levonorgestrel in the presence of the anti-androgen flutamide prevented anal fin elongation. > ability of levonorgestrel to induce anal fin elongation after a 21-day exposure to 0.1 µg/L (0.32 nM) levonorgestrel; Elongation was also present in female exposed to 0.5 g/L of TB > Mixture of levonorgestrel and trebolone there was no evident anal fin elongation compared to the control in females. During co-exposures of TB and mifepristone, anal fin elongation was significantly greater than the control	Transcriptomic and physiological changes in Eastern Mosquitofish (*Gambusia holbrooki*) after exposure to progestins andanti-progestagens	E.K. Brockmeier et al. / Aquatic Toxicology 179 (2016) 8–17

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
20	fathead minnow	progesterone (P4)	500 ng P4/L	in vitro exposure; transcriptional response in the ovary of fathead minnows	> Germinal vesicle migration and breakdown was observed and microarrays were used to identify gene cascades affected by P4. > Microarray analysis identified 1702 differentially expressed transcripts after P4 treatment. Functional enrichment analysis revealed that transcripts involved in the molecular functions of protein serine/threonine kinase activity, ATP binding, and activity of calcium channels were increased after P4 treatment. There was an overwhelming decrease in levels of transcripts of genes that are structural constituents of ribosomes with P4 treatment. > There was also evidence for gene expression changes in steroid and maturation-related transcripts. > Pathway analyses identified cell cycle regulation, insulin action, hedgehog, and B cell activation as pathways containing an over-representation of highly regulated transcripts. Significant regulatory sub-networks of P4-mediated transcripts included genes regulated by tumor protein p53 and E2F transcription factor 1.	Transcriptional signature of progesterone in the fathead minnow ovary (Pimephales promelas)	N. Garcia-Reyero et al. / General and Comparative Endocrinology 192 (2013) 159–169
21	Xenopus laevis, tadpoles stage 56	levonorgestrel	0.01–10 nM	> in vivo experiments with tadpoles and ex vivo culture of thyroid gland > exposure to 0.01, 0.1, 1 and 10 nM (corresponds to 3.124, 31.24, 312.4	> no treatment-related changes were observed in brain-pituitary tissue, LNG treatment readily affected thyroidal gene expression in tadpoles including decreased slc5a5 and iyd mRNA expression and a strong induction of dio2 and dio3 expression. > in ex vivo organ explant culture approach, direct effects of LNG on both pituitary and thyroid gland gene expression were detected, treatment of pituitary explants with 10 nM LNG strongly stimulated dio2 expression and concurrently suppressed tshb expression.	The synthetic gestagen levonorgestrel directly affects gene expression in thyroid and pituitary glands of Xenopus laevis tadpoles	C. Lorenz et al. / Aquatic Toxicology 177 (2016) 63–73

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
				and 3124 ng/L), for 72 h, brain-pituitary and thyroid tissue was analyzed for marker gene expression	> In thyroid glands, ex vivo LNG treatment induced dio2 and dio3 mRNA expression in a thyrotropin-independentmanner. > When thyroid explants were cultured in thyrotropin-containing media, LNG caused similar geneexpression changes as seen after 72 h in vivo treatment including a very strong repression of thyrotropin-induced slc5a5 expression. > ex vivo data provide clear evidence that LNG directly affects expression of genes important for thyroidal iodide handling as well as genes involved in negative feedback regulation of pituitary tshb expression.		
22	fathead minnow, larvae	levonorgestrel	125 ng/L	exposure up to 28 days post hatch (0, 3, 7, 14, and 28 dph), growth and mRNA expression of FSH, 3 -HSD, 20 -HSD, and CYP19a1 measured	> LNG significantly decreased growth in the fathead minnow larvae at day 28. > For both 20 -HSD and CYP19a1, mRNAexpression was decreased following LNG exposure durations ≥ 7 days. > 3 -HSD and FSH showed similar trends after exposure to LNG with later stages of development exhibiting decreased expression. > 20 -HSD and 3 -HSD were the only transcripts to remain down regulated once larvae were moved to clean water after the 7–14 dph LNG exposure	Responses to various exposure durations of levonorgestrel during early-life stages of fathead minnows (Pimephales promelas)	M.D. Overturf, D.B. Huggett / Aquatic Toxicology 161 (2015) 33–40
23	common carp, Cyprinus carpio	medroxyprogester one acetate	100 µg/L	exposure to 100 µg/L of MPA for a 7-day period followed by a depuration phase (14 days)	Tissue-specific bioconcentration factors (BCF) ranged from 4.3 to 37.8 and up take was greatest in the liver > brain > plasma and lowest in the muscle	Tissue-specific bioconcentration of the synthetic steroid hormone medroxyprogesteronea cetate in the common carp (Cyprinus carpio)	Steele, W. B. et al., (2103), Environmental Toxicology and Pharmacology 36 1120–1126

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24	zebrafish, adults and embryos	mifepristone (MIF, RU486), progesterone (P4)	5 - 77 ng/L MIF, 25 ng/L P4	adult zebrafish (F0) exposure for 21 days to 5, 39, 77 ng/L MIF, 25 ng/L P4 zebrafish embryos (F1) esposure for 96 hours to 3, 15, 26 ng/L MIF, and 254 ng/L P4	> significant U-shaped increase in egg production after exposure to 5 and 77 ng/L MIF, but no effects at 25 ng/LP4. > Levels of sex steroid hormones in blood plasma of adult males (11-ketotestosterone) and females (17-estradiol) were not altered. > In females increase of mature vitellogenic oocytes in ovaries exposed to MIF and P4, > several histopathological changes in ovaries, including post-ovulatory follicles, atretic follicles and proteinaceous fluid. > Male gonads showed no or less alterations andno histopathological effects. > Fertility of eggs and hatching success of embryos (F1 generation) was notaffected at 3–26 ng/L MIF and 254 ng/L P4, respectively	Effects of low concentrations of the antiprogestin mifepristone(RU486) in adults and embryos of zebrafish (Danio rerio): 1.Reproductive and early developmental effects	N. Blüthgen et al. / Aquatic Toxicology 144– 145 (2013) 83– 95
25	zebrafish adult and embryos	mifepristone (MIF, RU486), progesterone (P4)	5 - 77 ng/L MIF, 25 ng/L P4	adult zebrafish (F0) exposure for 21 days to 5, 39, 77 ng/L MIF, 25 ng/L P4, originated embyros were further exposed for 120 hpf. Gene expression analyses of adult tissue and zebrafish embyros Recombinant yeast-based assays (YES, YAS, YPS) and	> MIF elicited antiestrogenic, androgenic and progestogenic activities in recombinant yeast, similar to P4, and no antiprogestogenicactivity in vitro. > The transcriptional alterations of steroid hormone receptors were similar in adult males and females, and more pronounced in embryos. > MIF tended to transcriptionally down-regulate the androgen, progesterone and glucocorticoid receptors in adult fish and embryos. > Transcripts of the estrogen receptor (esr1) and vitellogenin (vtg1) were not significantly altered. > A trend for down-regulation was observed for transcripts of genes belonging to steroidogenic enzymes including 17 -hydroxysteroiddehydrogenase type 3 (hsd17b3), 3 -hydroxysteroid dehydrogenase (hsd3b), P450 aromatase A (cyp19a) and 11 -hydroxylase (cyp11b). P4 resulted in similar transcriptional	Effects of low concentrations of the antiprogestin mifepristone(RU486) in adults and embryos of zebrafish (Danio rerio): 2. Geneexpression analysis and in vitro activity	N. Blüthgen et al. / Aquatic Toxicology 144– 145 (2013) 96– 104

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
				compared to transcriptional alterations obtained in fish tissues and embryos from the exposure study.	alterations as MIF. > data indicates that gene expression changes and in vitro activities match only in part including the lack of antiprogestogenic activity of MIF		
26	zebrafish adult	megestrol acetate (MTA), 17α-ethinylestradiol (EE2)	10 -333 ng/L	MTA (33, 100 or 333 ng/L), EE2 (10 ng/L) or a mixture of both (MTA þ EE2: 33 þ 10, 100 + 10 or 333 + 10 ng/L) for 21 days	> egg production was significantly reduced by exposure to 10 ng/L EE2, but not MTA > combined exposure to MTA and EE2 caused further reduction of fish fecundity compared to EE2 exposure alone > Plasma concentrations of 17b-estradiol and testosterone in the females and 11-ketotestosterone in the males were significantly decreased in the groups exposed to EE2 or MTA alone, and were further reduced in the co-exposure groups > exposure to MTA and EE2 (alone or in combination) led to histological alterations in the ovaries (decreased vitellogenic/mature oocytes), but not in the testes	The binary mixtures of megestrol acetate and 17α-ethynylestradiol adversely affect zebrafish reproduction	J. Hua et al. / Environmental Pollution 213 (2016) 776e784
27	zebrafish	drospirenone (DRS)	27 - 5442 ng/L DRS	exposure to 55, 553, and 5442 ng/L DRS for 14 days; characterisation of global gene expression profiles of ovary and brain of female	> DRS and mixture treatments at the highest concentrations led to lower gonado-somatic index > exposure to 55, 553, and 5442 ng/L of DRS resulted in statistically significant differential expression of 38, 22, and 19 genes in the ovary > In the brain, transcriptomics analysis revealed 50, 64, and 109 differentially expressed genes in DRS, P4 and mixture (P4+DRS), irrespective of the dose, compared to controls > Multivariate analysis indicated tissue-, dose-, and treatment-dependent expression profiles.	Transcriptional and Physiological Responses Induced by Binary Mixtures of Drospirenone and Progesterone in Zebrafish (Danio rerio)	Zucchi et al 2014 / Environ. Sci. Technol. 2014, 48, 3523–3531

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				zebrafish were; effects were compared to the observed responses after exposure to mixtures of DRS and P4 (DRS+P4: 27 + 0.8, 277 + 8 and 3118 + 123 ng/L).	> Genes involved in steroid hormone receptor activity and circadian rhythm were enriched in DRS and mixture groups, among other pathways. In mixtures, the magnitude of response was dose- and transcript-dependent, both at the molecular and physiological levels. > Effects of DRS and P4 were additive for most of the investigated parameters and occurred at environmentally relevant concentrations.		
28	GR-CALUX bioassay	wastewaters and rivers in Czech Republic and Switzerland with 36 target compounds		Glucocorticoid receptor (GR)-mediated activity was determined using the GR-CALUX bioassay with dexamethasone equivalent concentrations ranging from <LOD−2.6, 19−37, and 78−542 ng/L for river water, treated, and untreated wastewater	>combined concentration of 17 assessed GCs in river water samples was in the range of 23−57 ng/L, with beta-/dexamethasone, prednisolone/prednisone and cortisol/cortisone being the most abundant GCs > For most samples, the chemically predicted GR-mediated response was higher than that determined by the bioassay. Correspondingly, antiglucocorticoid activity was observed in some fractions.	Endocrine Disrupting Compounds Affecting Corticosteroid Signaling Pathways in Czech and Swiss Waters: Potential Impact on Fish	Macikova 2014 / Environ. Sci. Technol. 2014, 48, 12902−12911
29	zebrafish, adult	progesterone (P4), drospirenone (DRS)	7 - 13´650 ng/L	Breeding pairs of adult zebrafish were	Of totally 10 key photo-transduction cascade genes analyzed, transcriptional levels of most were significantly upregulated, or normal down-	Progestins alter phototransduction cascade and circadian	Zhao 2016 / Scientific Reports | 6:21559

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				exposed to P4 and DRS for 21 days with different measured concentrations of 7–742 ng/L and 99-13´650 ng/L, respectively	regulation was attenuated. > Similarly, for some circadian rhythm genes, dose-dependent transcriptional alterations were also observed in the totally 33 genes analyzed. > Significant alterations occurred even at environmental relevant levels of 7 ng/L P4. Different patterns were observed for these transcriptional alterations, of which, the nfil3 family displayed most significant changes > photo-transduction signals and circadian rhythm are potential targets for progestins	rhythm network in eyes of zebrafish (Danio rerio)	
30	human, rainbowfish (Melanotaenia fluviatilis), zebrafish embryo	chlormadinone acetate (CMA), cyproterone acetate (CPA), 17α-ethinylestradiol (EE2),	1 -100000 ng/L	in vitro interaction with human and rainbowfish (Melanotaenia fluviatilis) sex hormone receptors, transcriptional alterations in zebrafish 96 and 144 hpf	> For human receptors, both progestins exhibited progestogenic, androgenic and antiestrogenic activity with no antiandrogenic or estrogenic activity. In contrast, interactions with rainbowfish receptors showed no progestogenic, but antiandrogenic, antiglucocorticoid, and some antiestrogenic activity > CMA led to slight down-regulation of the ar transcript, while CPA down-regulated ar and pgr transcripts. EE2 exposure resulted in significant transcriptional alterations of severalgenes, including esr1, pgr, vtg1, cyp19b, and gonadotropins (fshb, lhb). The mixture activity of CMA andEE2 followed the independent action model, while CPA and EE2 mixtures showed additive action intranscriptional alterations. > mixtures of CMA and CPA, and of CMA and EE2 behaved according to the concentration addition model in their in vitro interaction with human and rainbowfish receptors, often showing antagonism. In zebrafish embryos, binary mixtures of CMA and EE2 showed the same expression patterns as EE2 alone, indicating an independent action in vivo.	Effects of antiandrogenic progestins, chlormadinone and cyproterone acetate, and the estrogen 17α-ethinylestradiol (EE2), and their mixtures: Transactivation with human and rainbowfish hormone receptors and transcriptional effects in zebrafish (Danio rerio) eleuthero-embryos	P.F. Siegenthaler et al. / Aquatic Toxicology 182 (2017) 142–162

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
31	Three-Spined Stickleback (Gasterosteus aculeatus)	levonorgestrel (LNG)	5.5 - 358 ng/L	exposure of adult female sticklebacks to LNG at 5.5, 40, and 358 ng/L for 21 days.	> Androgenic effects were found at LNG concentrations ≥40 ng L−1 including induction of spiggin transcription, kidney hypertrophy, and suppressed liver vitellogenin transcription. > Histological evaluation of the kidneys showed that exposure to LNG at 40 and 358 ng L−1 caused a significant increase of the height of the proximal tubule epithelium > induction of kidney spiggin transcription at 40 and 358 ng L−1 > A dose-dependent down-regulation of liver vitellogenin transcription at 40 ng L−1 (p = 0.0191) and 358 ngL/1 was observed	The Synthetic Progestin Levonorgestrel Is a Potent Androgen in the Three-Spined Stickleback (Gasterosteus aculeatus)	Svensson et al./ Environ. Sci. Technol. 2013, 47, 2043−2051
32	Three-Spined Stickleback (Gasterosteus aculeatus)	levonorgestrel (LNG)	nominal 10, 100 and 1000 ng/L (measured conc. 6.5, 65and 750 ng/L)	Male sticklebacks in the final stage of a breeding period were exposed to various concentrations of levonorgestrel for six weeks under winter conditions	> controls had transitioned from full breeding condition into the non-breeding state, including regression of secondary sex characteristics, cessation of spiggin production in the kidney, and resumption of spermatogenesis in the testes. > groups exposed to levonorgestrel, transition to the non-breeding condition was dose-dependently inhibited. >nephrosomatic index decreased significantly during the exposure period in the control group and the group exposed to 6.5 ng/L compared with the initial controls, but was maintained by LNG at ≥65 ng/L > Results show that levonorgestrel can disrupt the seasonal breeding cycle in male sticklebacks. The fitness costs of such an effect could be detrimental to natural stickleback populations. >Some effects occurred at a levonorgestrel concentration of 6.5 ng L, well within the range of levonorgestrel levels in surface waters	Environmental concentrations of an androgenic progestin disruptsthe seasonal breeding cycle in male three-spined stickleback (Gasterosteus aculeatus)	J. Svensson et al. / Aquatic Toxicology 147 (2014) 84– 91

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
33	fathead minnow, adult	17 - ethinylestradiol, levonorgestrel	0.5, 5 and 25 ng/lof either EE2 or LNG mixture study with a fix ratio (1:1) of the chemicals,with concentrations of 0.25, 2.5 and 12.5 ng/l (combined concentrations were 0.5, 5 and 25 ng/l)	21 day ‘pair-breeding’ assay to assess reproductive output	> egg production,which was inhibited in a concentration-dependent manner by both the individual chemicals and the mixture (LNG EC20 of 0.9 ng/l; EE2 EC20 = 0.4 ng/l) > LNG exposure lead to sex specific effects; while there no sig. Effects on males, there were effect on females such as, conc. dependent increase in weight, lenght and secondary sex characterisitcs. > EE2 exposure increased sig. the plasma vitellogenin concentration in females; stronger effects on males: increased ovipositorlength, reduced the prominence of secondary sexual characteris-tics such as tubercle number > The mixture of EE2 and levonorgestrel significant effects on both sexes	From single chemicals to mixtures—Reproductive effects oflevonorgestrel and ethinylestradiol on the fathead minnow	T.J. Runnalls et al. / Aquatic Toxicology 169 (2015) 152–167
34	fathead minnow	levonorgestrel	10-100 ng/L	Adult females were exposed to 0, 10, or 100 ng/L levonorgestrel for 14 d	> Females exposed to both concentrations of levonorgestrel developed male secondary sexual characteristics in a dose-dependent manner, and ovaries contained significantly fewer late stage oocytes. Exposure to 100 ng/L of levonorgestrel resulted in decreased GSI and blood plasma vitellogenin concentrations.	Exposure effects of levonorgestrel on oogenesis in the fathead minnow (Pimephales promelas)	Frankel et al., Environ Toxicol Chem 2017; 9999: 1–6
35	sea lamprey (Petromyzon marinus)	progesterone (P), 15α-hydroxyprogesterone (15a-P)		> determine the differences in mRNA expression levels of nuclear progestin receptor (nPR) and the membrane receptor adaptor	> Expression levels of nPR and pgrmc1 differed between life stages and tissues, and in some cases were differentially responsive to lamprey GnRH-I and -III. > Increases in nPR and pgrmc1 gene expressions were correlated to the late stages of sexual maturation in males. The highest expression levels of these genes were found in the liver and gill of spermiating males. >These organs are, respectively, the site of production and release of the sex pheromone 3 keto-petromyzonol sulfate (3kPZS). The hypothesis	Evidence that progestins play an important role in spermiation and pheromone production in male sea lamprey (Petromyzon marinus)	M.B. Bryan et al. / General and Comparative Endocrinology 212 (2015) 17–27

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				protein 'progesterone receptor membrane component 1' (pgrmc1) in putative target tissues in males at different life stages > to demonstrate the function of progestins by implanting prespermiating males (PSM) with time-release pellets of P and measuring the latency to the onset of spermiation and plasma concentrations of sex pheromones and steroids. >to measure the binding affinity of P in the nuclear and membrane	that pheromone production may be under hormonal control was tested in vivo by implanting PSM with time-release pellets of P. Concentrations of 3kPZS in plasma after 1 week were 50-fold higher than in controls or in males that had been implanted with androstenedione, supporting the hypothesis that P is responsible for regulating the production of the sex pheromone. >P treatment also accelerated the onset of spermiation. Saturation and Scatchard analyses of the target tissues showed that both nuclear and membrane fractions bound P with high affinity and low capacity (KD 0.53 pmol/g testis and 0.22 pmol/g testis, and Bmax 1.8 and 5.7 nM, respectively), similar to the characteristics of nPR and mPR in other fish.		

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				fractions of the target tissues			
36	fathead minnows (Pimephales promelas)	progesterone-spiked sand and silty loam sediment		fate of progesterone in two natural sediments and the corresponding alteration of gene expression in three steroid-responsive genes; vitellogenin, androgen receptor and estrogen receptor alpha	> When exposed to progesterone-spiked sand, fathead minnows exhibited significant reductions in the expression of vitellogenin and androgen receptor expression. > In contrast, fish exposed to progesterone associated with the silty loam sediment did not show a biological response at 7 days and only realized a significant reduction in vitellogenin. > In both sediments, progesterone degradation resulted in the production of androgens including androsteinedione, testosterone, and androstadienedione, as well as the antiestrogen, testolactone	Bioavailability and Fate of Sediment-Associated Progesterone in Aquatic Systems	Sangster et al 2016 / Environ. Sci. Technol. 2016, 50, 4027−4036 DOI: 10.1021/acs.est.5b 06082
37	zebrafish, female	progesterone	3.5, 33 and 306 ng/L P4	exposure of female zebrafish exposed for 14 days global expression profile in the brain and ovary	> In the brain, 54 and 255 transcripts were altered at 3.5 and 306 ng/L, respectively. >Genes related to circadian rhythm (nr1d2b, per1b), cell cycle and reproduction (cdc20, ccnb1) were down-regulated. > In the ovary, transcriptional changes occurred in 200, 84 and 196 genes at 3.5, 33 and 306 ng/L, respectively. The genes belong to different pathways including cardiac hypertrophy, cell cycle and its regulation. P4 slightly influenced oocyte maturation as revealed by histology of the ovaries. > In the liver, vtg1 was down-regulated at all concentrations and VTG protein at 306 ng/L in the blood.	Progesterone Alters Global Transcription Profiles at Environmental Concentrations in Brain and Ovary of Female Zebrafish (Danio rerio)	Zucchi et al./ dx.doi.org/10.1021 /es403800y / Environ. Sci. Technol. 2013, 47, 12548−12556

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38	zebrafish embryos	progesterone (P4), norgestrel (NGT)	5, 50 and 100 ng L−1	exposure for 144 h post fertilization; transcriptional levels of target genes along the hypothalamic–pituitary–thyroid axis were determined daily	> The results showed that P4 had only minor effects on themRNAexpression of thyroglobulin (Tg), iodothyronine deiodinase type I (Dio1) and thyroid hormone receptor β (Thrb) genes. > the effects of NGT on transcripts of thyrotropin-releasing hormone (Trh), Dio1, iodothyronine deiodinase type II (Dio2) and thyroid hormone receptor α (Thra) genes were generally low. > NGT resulted in some alterations of Tg and Thrb transcripts at different time points. However, a strong induction of Nis mRNA by P4 and NGT was observed in zebrafish embryos-larvae. > overall results showed that besides Nis no effects on the hypothalamic–pituitary–thyroid (HPT) axis are observed following exposure to P4 and NGT, which imply that both P4 and NGT have potential effects on the thyroid endocrine system by inducing transcript of Nis gene during the early stage of zebrafish	Progesterone and norgestrel alter transcriptional expression of genes along the hypothalamic–pituitary–thyroid axis in zebrafish embryos-larvae	Y.-Q. Liang et al. / Comparative Biochemistry and Physiology, Part C 167 (2015) 101–107
39	fathead minnow, nuclear PR, in vitro	17α,20β-dihydroxy-4-pregnen-3-one (DHP), 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S), progesterone (P4)	environmentally relevant	to develop an in vitro assay for FHM nPR transactivation , and to screen eight gestagens for their ability to transactivate fathead minnow nPR. Also, the ability of these gestagens to transactivate FHM androgen	> Fish progestogens activated FHM nPR, with DHP being more potent than 20β-S. > The progestin drospirenone and P4 transactivated the FHM nPR, whereas five progestins and P4 transactivated FHM AR, all at environmentally relevant concentrations. > several progestins proved to be strong agonists of AR. > mechanistic evidence that environmental gestagens can activate fathead minnow nPR and AR, suggesting that gestagens may affect phenotype through nPR- and AR-mediated pathways	Environmental Gestagens Activate Fathead Minnow (Pimephales promelas) Nuclear Progesterone and Androgen Receptors in Vitro	Ellestad 2014 / dx.doi.org/10.1021 /es501428u | Environ. Sci. Technol. 2014, 48, 8179−8187

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				receptor (AR) was investigated			
40	fathead minnow	progesterone (P4)	10- 1000 ng/L	reproduction assay: mature fish were exposed for 21 d to nominal concentrations of 0, 10, 100, and 1,000 ng/L P4 in a flow-through system; embryonic development assay: effects of P4 on early development and hatching success	> Progesterone caused dose-dependent decreases in fecundity and fertility and significantly reduced gonadosomatic index and vitellogenin gene expression in females. > There were no effects of P4 on early embryonic development or hatching success.	Effects of progesterone on reproduction and embryonic development in the fathead minnow (Pimephales promelas)	De Quattro et al. / Environ. Toxicol. Chem. 2012;31:851–856. © 2012 SETAC / DOI: 10.1002/etc.1754
41 / 72	fathead minnows, adult female	norethindrone, progesterone	progesteron 34 ± 4.1 ng/L; norethindrone 168 ± 7.5 ng/L	Effects on on steroidogenesis in adult female	>In vivo exposure to either compound lowered expression (nonsignificant) of luteinizing hormone (LHb) levels in the brain along with significantly down-regulating the beta isoform of membrane progesterone receptor (mPRb) in ovary tissue. >The correspondence between lowered LHb levels in the brain and mPRb in the ovary is suggestive of a possible functional association as positive correlations between LHb and mPR levels have been demonstrated in other fish species. > In vitro exposure of ovary tissue to progesterone resulted in significantly elevated progestogen	Effects of progesterone and norethindrone on female *Fathead minnow* (*Pimephales promelas*) steroidgenesis	L.H. Petersen et al. / Environ Toxicol Chem 34, 2015

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					(pregnenolone, 17a-hydroxyprogesterone, and 17a,20b-dihydroxypregnenone) and androgen (testosterone) production. >Whereas in vitro exposure to norethindrone did not significantly impact steroid hormone production but showed decreased testosterone production relative to solvent control (however this was not significant		
42	fathead minnow	progesterone (P4)	10^-6 M	ovary explants exposure for 6 and 12h, effects of P4 on testosterone (T) and 17b-estradiol (E2) production in the fathead minnow (FHM) ovary and on the mRNA abundance of transcripts involved in steroidogenesis and steroid receptor signaling.	> P4 administration significantly increased T production 3-fold at both 6 and 12 h, whereas E2 production was not affected > Nuclear progesterone receptor mRNA was decreased at 6 h and membrane progesterone receptor gamma-2 mRNA was significantly down-regulated at both 6 and 12 h > no change in membrane progesterone receptor alpha or beta mRNA levels > Androgen receptor (ar) and estrogen receptor 2a (esr2a) mRNA were significantly reduced at 6 h with P4 treatment, but there was no change in esr2b mRNA at either time point. >Transcripts for enzymes in the steroid pathway (star, hsd11b2) were significantly lower at 6 h compared to controls, whereas cyp17a and cyp19a mRNA abundance did not change with treatments at either time point.	Progesterone increases ex vivo testosterone production and decreases the expression of progestin receptors and steroidogenic enzymes in the fathead minnow (Pimephales promelas) ovary	Chishti et al. / General and Comparative Endocrinology 199 (2014) 16–25 http://dx.doi.org/10.1016/j.ygcen.2014.01.004
43	eastern mosquitofish (Gambusia holbrooki)	levonorgestrel	10 - 100 ng/L	exposure of adult mosquitofish to ethanol (EtOH control), 10 ng/L, and	> Female mosquitofish exposed to LNG were masculinized as evidenced by the elongation of the anal fin rays > LNG caused significant increases in the 4:6 anal fin ratios of female mosquitofish in both the 10 ng/L and 100 ng/L treatments, although these	Aqueous exposure to the progestin, levonorgestrel, alters anal fin development and reproductive behavior	Frankel et al. / General and Comparative Endocrinology 234 (2016) 161–169 http://dx.doi.org/1

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				100 ng/L LNG for 8 d using a static replacement exposure design	differences were not significant between the two treatments. > LNG caused significant increases in the 4:6 anal fin ratio of males exposed to 100 ng/L, with no effects observed in the 10 ng/L treatment. > the reproductive behavior of control males paired with female mosquitofish exposed to 100 ng/L LNG was also altered	in the eastern mosquitofish (Gambusia holbrooki)	0.1016/j.ygcen.2016.01.007
44	fathead minnow	levonorgestrel	5 - 3125 ng/L	OECD 210 ELS, 28 days post-hatch; specific endocrine related mRNA transcript profiles in FHM larvae; ex vivo steroidogenesis assay was conducted with sexually mature female FHM following a7 day exposure 100 ng/L LNG	> survival of larvalFHM was impacted at 462 ng/L, while growth was significantly reduced at 86.9 ng/L. > Transcripts of 3 -HSD, 20 -HSD, CYP17, AR, ER , and FSH were significantly down-regulated following 28 d exposure to 16.3 ng/L LNG, while exposure to 86.9 ng/L significantly down-regulated 3 -HSD, 20 -HSD, CYP19A, and FSH. At 2392 ng/L of LNG, a significant down-regulation occurred with CYP19A and ER transcripts, while mPR and mPR profiles were significantly induced > No significant changes occurred in 11 -HSD, CYP11A, StAR, LH , and VTG mRNA expression following LNG exposure > steroidgenesis assay: significant reductions observed in pregnenolone, 17 ,20 -dihydroxy-4-pregnen-3-one (17,20-DHP), testosterone, and 11-ketotestosterone.	Levonorgestrel exposure to fathead minnows (Pimephales promelas) alters survival, growth, steroidogenic gene expression andhormone production	Overturf et al. / Aquatic Toxicology 148 (2014) 152–161 http://dx.doi.org/10.1016/j.aquatox.2014.01.012
45	zebrafish, embryo	dydrogesterone	50 – 5000 ng L^{-1}	eleuthero-embryos were analyzed for the transcriptional alterations by real-time quantitative PCR (RT-qPCR)	> The results of qPCR analysis showed that DDG exposure significantly suppressed the transcriptions of target genes involved in hypothalamic–pituitary–thyroid (HPT) axis, while it induced the expression of target genes mRNA belonging to hypothalamic–pituitary–gonad (HPG) axis. >ATR-FTIR spectroscopy analysis showed that the biochemical alterations of protein, nucleic acid and lipid were observed following DDG treatment.	Transcriptional and Biochemical Alterations in Zebrafish Eleuthero-Embryos (Danio rerio) After Exposure to Synthetic Progestogen Dydrogesterone	Shi et al / Bull Environ Contam Toxicol (2017) 99:39–45 DOI 10.1007/s00128-017-2046-1

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				and biochemical changes by attenuated total reflection Fouriertransform infrared spectroscopy (ATR-FITR) after 144 h exposure to DDG.			
46	Xenopus laevis	levonorgestrel (LNG), progesterone (PRG)	10^{-7} - 10^{-10} M	mating behavior of male Xenopus laevis	> LNG at all exposure concentrations increased the proportions of advertisement calling, indicating a sexually aroused state of the males > LNG at 10^-7 M decreased the relative proportions of rasping, a call type indicating a sexually unaroused state of the male > PRG did not affect any of those parameters	The synthetic progestogen, Levonorgestrel, but not natural progesterone, affects male mate calling behavior of Xenopus laevis	Hoffmann et al / General and Comparative Endocrinology 176 (2012) 385–390 / doi:10.1016/j.ygcen.2012.02.009
47	zebrafish	levonorgestrel	10 ngL^-1 and 1000 ngL^-1	exposure for 21-days to LNG and manipulation of temperature of +3°C.	>Increased temperature caused an overall decrease in the females' gonadosomatic index (GSI), during the pre-reproduction phase, LNG did not affect GSI > fecundity was negatively affected by both temperature and LNG > Fish exposed to the highest LNG concentration (at both temperatures) did not reproduce > also fish exposed to the lowest dose of progestin at a higher temperature, a complete reproductive failure occurred. > 10 ngL-1 of LNG (at 27 C) reduced significantly the hatching rate, comparing to control	Warming modulates the effects of the endocrine disruptor progestin levonorgestrel on the zebrafish fitness, ovary maturation kinetics and reproduction success	Cardoso et al. / Environmental Pollution 229 (2017) 300e311 http://dx.doi.org/10.1016/j.envpol.2017.05.090
48	zebrafish	norgestrel	6, 29 and 69 ng L-1 NGT	exposure of adult zebrafish for 21 days	>exposure to 69 ng L 1 NGT led to a significant up-regulation of follicle stimulating hormone, beta polypeptide (fshb), luteinizing hormone, beta	Reproductive effects of synthetic progestin	Liang et al. / Chemosphere 190 (2018) 17-24

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					polypeptide (lhb), progesterone receptor (pgr), estrogen receptor 1 (esr1) and androgen receptor (ar) genes in the brains, as well as significant upregulation of hydroxysteroid 20-beta dehydrogenase (hsd20b) and hydroxysteroid 11-beta dehydrogenase 2 (hsd11b2) genes and down-regulation of 11-beta-hydroxylase (cyp11b) gene in the ovaries of females. > In the testes of males, an overall down-regulation of steroidogenic acute regulatory protein (star), cytochrome P450-mediated side-chain cleavage enzyme (cyp11a1), cyp11b, hsd20b, hydroxysteroid 17-beta dehydrogenase type 3 (hsd17b3), hsd11b2 and ar genes were observed following exposure to different treatments of NGT. > These transcriptional alterations imply that NGT could exhibit the potent progestogenic and androgenic activities in zebrafish. > Egg production as well as histology in the ovaries and testes was not affected by NGT.	norgestrel in zebrafish (Danio rerio)	
49	rainbow trout	effluents		Rainbow trout were exposed to undiluted, treated sewage effluents at three sites in Sweden for 14 days	> The progestin pharmaceutical levonorgestrel was detected in fish blood plasma at concentrations (8.5-12ng mL-1) > In total 16 pharmaceuticals were detected in fish plasma at concentrations higher than 1/1000 of the human therapeutic plasma concentration	Therapeutic Levels of Levonorgestrel Detected in Blood Plasma of Fish: Results from Screening Rainbow Trout Exposed to Treated Sewage Effluents	Fick et al / Environ. Sci. Technol. 2010, 44, 2661–2666
50	fathead minnow	levonorgestrel (LNG), drospirenone (DRSP)	levonorgestrel 0.8, 3.3, and 29.6 ng/L, drospirenone 0.66, 6.5, and 70 µg/L	21-d fish reproduction screening assay	> Both tested progestins caused an inhibition of reproduction > For LNG, this occurred at concentrations of 2:0.8 ng/L > Higher concentrations resulted in masculinization of females with de novo synthesis of nuptial	Effects of synthetic gestagens on fish reproduction	Zeilinger et al 2009 / Environmental Toxicology and Chemistry, Vol. 28, No. 12, pp. 2663–2670, 2009

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					tubercles > Drospirenone treatment, however, affected the reproductive success of fathead minnow at concentrations of 6.5 µg/L and higher		DOI: 10.1897/08-485.1
51	Murray–Darling rainbowfish (Melanotaenia fluviatilis)	19-nortestosterone-derived progestins : etonogestrel, gestodene,norgestimate, levonorgestrel, 19-norethindrone, drospirenone, dihydrotestosterone; 17a20b-dihydroxy-4-pregnen-3-one; progesterone		transactivation assays driven by rainbowfish ARa, ARb and PR	> all tested progestins were highly potent agonists of rainbowfish ARa > only four of the five progestins were potent agonists of rainbowfish Arb > drospirenone, was not an agonist of rainbowfish ARa or ARb but was weak agonist of rainbowfish PR	Nortestosterone-derived synthetic progestogens do not activate the progestogen receptor of Murray–Darling rainbowfish (Melanotaenia fluviatilis) but are potent agonists of androgen receptors alpha and beta	Bain et al 2015 / Aquatic Toxicology 163 (2015) 97–101
52	fathead minnow, Japanese medaka	norethindrone		28 day static-renewal reproduction study with Japanese medaka; 21 day flowthough with fathead minnow reproduction study	> Japanese medaka indicated that NET produces a significant decrease in fecundity at aqueous concentrations ≥ 25 ng/L > fathead minnow reproduction study also demonstrated that NET causes a significant decrease in fecundity in the low ng/L range > Fathead minnow morphological changes (i.e. female fin spots) suggest that NET exposure may have a potent androgenic effect on fish; > plasma 11-Ketotestosterone (11-KT) concentrations were reduced in males at the highest exposure concentration	Reproductive responses in fathead minnow and Japanese medaka following exposure to a synthetic progestin, Norethindrone	Paulos et al / Aquatic Toxicology 99 (2010) 256–262
53	Scenedesmus obliquus,	progesterone, norgestrel		Degradation of progesterone and norgestrel	> More than 95% of progesterone was transformed by the two microalgae within 5 d > For norgestrel, almost complete transformation	Biotransformation of progesterone and norgestrel by two	F.-Q. Peng et al. / Chemosphere 95 (2014) 581–588

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	Chlorella pyrenoidosa			by two algae was investigated	by S. obliquus was observed after 5 d, but nearly 40% was remained when incubated with C. pyrenoidosa > The results also showed that these two compounds were not accumulated in the algal cells.	freshwater microalgae (Scenedesmus obliquus and Chlorella pyrenoidosa): Transformation kinetics and products identification	
54	male fathead minnow	progesterone (P4)	0, 30, and 300 ng/L	1-week *in vivo* exposure; short-term in vitro exposure Sperm were then video recorded and motility was analyzed by CASA	>continuous exposure for 1 week to low levels of progesterone in vivo caused a significant dose-dependent reduction in sperm motility. >no effect of short-term P4 exposure on fathead minnow sperm swimming characteristics.	Effects of progesterone on sperm motility in fathead minnow (Pimephales promelas)	P.J. Murack et al. / Aquatic Toxicology 104 (2011) 121–125
55	in vitro assays	levonorgestrel served as the reference compound		REVIEW on suitability of in vitro assays to detect endocrine activity in water	>While both the progesterone receptor binding assay and the yeast reporter gene assay YPH500-hPR-Gal assay used P4 as the reference compound, the MDLs from the reevaluated concentration effect curves were 340 and 3 ng/L, respectively >The HG5LN-Gal4-PR assay, which used PMG as the reference compound, was much less sensitive than the other mammalian reporter gene assays. > A MDL of 6.7 ng/L levonorgestrel equivalents (LevoEQ) was derived for PR-CALUX, while the MDL for PR-GeneBLAzer was 0.5 ng/L LevoEQ.	Analysis of the sensitivity of in vitro bioassays for androgenic, progestagenic, glucocorticoid, thyroid and estrogenic activity: Suitability for drinking and environmental waters	F.D.L. Leusch et al. / Environment International 99 (2017) 120–130
56				REVIEW	> Androgenic/progestagenic relative potencies or relative binding affinity of these SOCs as well as their physicochemical properties and toxicity are summarized.	Occurrence, fate and removal of synthetic oral contraceptives (SOCs) in the natural environment: A review	Z. Liu et al. / Science of the Total Environment 409 (2011) 5149–516

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57				REVIEW	> predicted environmental concentrations (PECs) suggest that if parent gestagens are expected to be found in the ng l−1 range, some active metabolites could be present at higher concentrations, although limited data on metabolism and environmental fate limit the relevance of PECs. > The biological effects are not expected to be restricted to progestagenic activity. > Both anti-androgenic activity (mainly for cyproterone acetate, chlormadinone acetate and their metabolites) and estrogenic activity (mainly for reduced metabolites of levonorgestrel and norethisterone) should also occur. >All these molecules are likely to have a cumulative effect among themselves or with other xenoestrogens.	Progestagens for human use, exposure and hazard assessment for the aquatic environment	J.-P. Besse, J. Garric / Environmental Pollution 157 (2009) 3485–3494
58				In this study, nine androgens, nine progestogens, and five estrogens were analyzed in influent and final effluent wastewaters in seven wastewater treatment plants (WWTPs) of Beijing, China	>Androgens contributed 96% of the total hormone concentrations in all WWTP influents, with natural androgen (androsterone: 2977 ± 739 ng/L; epiandrosterone: 640 ± 263 ng/L; and androstenedione: 270 ± 132 ng/L) being the predominant compounds. >The concentrations of synthetic progestogens (megestrol acetate: 41 ± 25 ng/L; norethindrone: 6.5 ± 3.3 ng/L; and medroxyprogesterone acetate: 6.0 ± 3.2 ng/L) were comparable to natural ones (progesterone: 66 ± 36 ng/L; 17α,20β-dihydroxy-4-progegnen-3-one: 4.9 ± 1.2 ng/L; 21α-hydroxyprogesterone: 8.5 ± 3.0 ng/L; and 17α-hydroxyprogesterone: 1.5 ± 0.95 ng/L)	Occurrence of androgens and progestogens in wastewater treatment plants and receiving river waters: Comparison to estrogens	Chang et al. / water research 45 (2011) 732-740

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59				wastewater and surface water samples from Japan	> The method detection limits for the eighteen analytes in the influent, effluent and surface water samples were 0.20–50, 0.04–20 and 0.01–12 ng/L, respectively. > This method was used to analyze the residual androgens and progestogens in the wastewater and surface water samples from Japan, and ten analytes (0.03 (medroxyprogesterone acetate)–1441 ng/L (androsterone)) were detected in the wastewater samples, and four analytes (0.06 (progesterone)–0.46 ng/L (androstenedione)) were detected in the surface water samples.	Trace analysis of androgens and progestogens in environmental waters by ultra-performance liquid chromatography–electrospray tandem mass spectrometry	Chang et al. /Journal of Chromatography A Volume 1195, Issues 1–2, 27 June 2008, Pages 44-51
60	Xenopus tropicalis	Levonorgestrel (LNG)	0.06, 0.5 nM	Tadpoles were exposed from hatching until metamorphosis. Remaining animals were held unexposed for nine months,	>LNG exposure severely impaired oviduct and ovary development and fertility. All adult females in the 0.5 nM group (n = 10) completely lacked oviducts. > They also displayed a significantly larger fraction of immature oocytes, arrested in meiotic prophase, than control females. >Upon mating with unexposed males, only one of 11 LNG-exposed females laid eggs, whereas all control females did. >No effects on testicular development, sperm count or male fertility were observed. >At metamorphosis, no effects on sex ratio or gonadal histology were evident. The effects on ovarian and oviductal development were detected at adult age but not at metamorphosis, emphasising the importance of investigating the long-term consequences of developmental exposure.	Early life progestin exposure causes arrested oocyte development, oviductal agenesis and sterility in adult Xenopus tropicalis frogs	M. Kvarnryd et al. / Aquatic Toxicology 103 (2011) 18–24
61	Dreissena polymorpha	Levonorgestrel (LNG)	0.312, 3.12 and 6.24 µg L−1	7 day exposure	> The lowest concentration (0.312 µg L−1) was 100-fold bioconcentrated within four days. >A decrease of the bioconcentration factor was observed within one week for the highest test concentrations (3.12 and 6.24 µg L−1) suggesting	Molecular effects and bioaccumulation of levonorgestrel in the non-target organism Dreissena polymorpha	V. Contardo-Jara et al. / Environmental Pollution 159 (2011) 38e44

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					enhanced excretory processes. >The immediate mRNA up-regulation of pi class glutathione S-transferase proved that phase II biotransformation processes were induced. >Disturbance of fundamental cell functions was assumed since the aryl hydrocarbon receptor has been permanently down-regulated. mRNA up-regulation of P-glycoprotein, superoxide dismutase and metallothioneine suggested enhanced elimination processes and ongoing oxidative stress. >mRNA up-regulation of heat shock protein 70 in mussels exposed to the two highest concentrations clearly indicated impacts on protein damage.		
62	Cyprinus carpio	synthetic and natural progestogens and the glucocorticoid cortisol		effects on leucocytes isolated from their kidneys	>Injection of cortisol led to an increased spleeno-somatic index (IS) on day 21 post-injection (pi) and immunosuppressive effects measured as decreased nitric oxide (NO) production and increased arginase activity in isolated leucocytes on days 14 and 21 pi, respectively. >reduced NO production was also observed after injection of the synthetic progestogens, levonorgestrel (LEV) and medroxyprogesterone acetate. >In addition, LEV influenced arginase activity in head kidney cells on day 14 and day 21 pi.	In vivo treatment with progestogens causes immunosuppression of carp Cyprinus carpio leucocytes by affecting nitric oxide production and arginase activity	C. PIETSCH ET AL. / Journal of Fish Biology (2011) 79, 53–69
63	zebrafish embryo	Drospirenone (DRS), progesterone (P4), medroxyprogester one acetate (MPA) and dydrogesterone (DDG)	exposure to single compounds and to binary mixtures	in vitro in recombinant yeast assays with the human progesterone, androgen, or estrogen receptor; in	> in vitro: Mixtures of DRS and P4, as well as of DRS and EE2 showed additive progestogenic and androgenic activities. However, DDG and MPA showed non-additive progestogenic and androgenic activities. > in vivo: DRS, P4, and EE2 led to significant transcriptional alteration of genes, including those encoding hormone receptors (pgr, esr1), a steroidogenic enzyme (hsd17b3), and estrogenic	Activity of binary mixtures of drospirenone with progesterone and 17α-ethinylestradiol in vitro and in vivo	N.M. Rossier et al. / Aquatic Toxicology 174 (2016) 109–122

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				vivo in zebrafish embryos	markers (vtg1, cyp19b), in particular at 144 hpf. In general, DRS showed stronger transcriptional changes than P4. In mixtures of DRS and P4, they were mainly non-additive (antagonistic interaction). In mixtures of DRS and EE2, transcriptional responses of esr1, vtg1 and cyp19b were dominated by EE2, suggesting an antagonistic interaction or independent action. Equi-effective mixtures of DRS and EE2, based on progesterone receptor transcripts, showed antagonistic interactions.		
64	zebrafish embryo	artificial mixture representing wastewater concentrations		hospital waste waters, river water, and municipal wastewater treatment plant (WTP) influents and effluents at different sites in Switzerland	>Considerable levels of different steroids occurred in hospital and raw municipal wastewater, but they were low (lower than 1 ng/L) or below the detection level in effluents of WTPs and river water. >In WTP influents, estrogens (estrone, 17β-estradiol, and estriol), androgens (androstenedione, androsterone, trans-androsterone, and testosterone), progestins and metabolites (progesterone, medroxyprogesterone acetate, megestrol acetate, mifepristone, pregnanediol, 17α-hydroxypregnanolone, 17α-hydroxyprogesterone, and 21α-hydroxyprogesterone) were detected and removed effectively during biological treatment. >Ozonation further removed the steroids. Exposure of zebrafish embryos demonstrated negligible effects of pregnanediol and 17α-hydroxypregnanolone, while mixtures that mimic wastewater and river water composition affected embryo development and led to the alteration of steroidogenesis gene transcripts at nanogram per liter concentrations.	Occurrence and Ecotoxicological Effects of Free, Conjugated, and Halogenated Steroids Including 17α-Hydroxypregnanolone and Pregnanediol in Swiss Wastewater and Surface Water	Zhang et al / Environ. Sci. Technol., 2017, 51 (11), pp 6498–6506 DOI: 10.1021/acs.est.7b01231

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
65	Mytilus galloprovincialis	Drospirenone (DRO)	20-10.000 ng/L	exposure for 7 days	>Following exposure, no significant effect was observed on gonad maturation of treated and untreated mussels. >The levels of progesterone (P4) and testosterone (T) were measured in mantle/gonad tissues and no significant alteration detected after exposure. >However, the application of a protonic nuclear magnetic resonance (1H NMR)-based metabolomics approach enabled a comprehensive assessment of DRO effects in mussels. Specifically, 1H NMR metabolic fingerprints of digestive glands of DRO treated mussel groups were clearly separated from each other and from controls through a principal component analysis (PCA). >Moreover, a number of metabolites involved in different metabolic pathways were found to significantly change in DRO-exposed mussels compared to control, suggesting the occurrence of alterations in energy metabolism, amino acids metabolism, and glycerophospholipid metabolism.	Sex steroids and metabolic responses in mussels Mytilus galloprovincialis exposed to drospirenone	T. Cappello et al. Ecotoxicology and Environmental Safety 143 (2017) 166–172
66	Rutilus rutilus, adult	mixture of progesterone, drospirenone, and levonorgestrel	10 -500 ng/L	exposure to mixtures for 42 days	>The somatic index of liver and kidney significantly in all the treated groups, whereas the gonadosomatic index of 500 ng/L treated group showed significant decrease compared to control animals. >VTG level increased significantly in 500 ng/L progestogen treated group. > Since the concentration of DJ-1 significantly increased in brain and liver in all progestogen treatment groups, the DJ-1 protein could be able to a more sensitive marker than VTG. >Serum LDL and cholesterol levels of exposed fish were significantly decreased. DJ-1 was mediated through the stimulation of the expression of LDL-receptor which facilitates reuptake subsequently.	Complex molecular changes induced by chronic progestogens exposure in roach, Rutilus rutilus	G. Maasz et al. / Ecotoxicology and Environmental Safety 139 (2017) 9–17

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
67	Lymnaea stagnalis	progesterone, levonorgestrel, drospirenone, and gestodene	10 ng/L	exposed to a mixture of four progestogens for 3 weeks	>The treatment of adult Lymnaeas caused reduced egg production, and low-quality egg mass on the first week, compared to the control. >Starting from the second week, the egg production, and the quality of egg mass were similar in both groups. >At the end of the third week, the egg production and the vitellogenin-like protein content of the hepatopancreas were significantly elevated in the treated group. >At the cellular level, accelerated cell proliferation was observed during early embryogenesis in the treated group. The investigation of metabolomic changes resulted significantly elevated hexose utilization in the single-cell zygote cytoplasm, and elevated adenylate energy charge in the egg albumen. > These changes suggested that treated snails provided more hexose in the eggs in order to improve offspring viability.	Effect of progesterone and its synthetic analogs on reproduction and embryonic development of a freshwater invertebrate model	Z. Zrinyi et al. / Aquatic Toxicology 190 (2017) 94–103
68	Gambusia affinis	Progesterone (P4)	4-410 ng/L	exposure of adult fish for 42 days	>The expression levels of genes (vtg, er, and ar isoforms) related to fish reproduction and detoxification (cyp1a) in the liver were quantified by quantitative real-time polymerase chain reaction. >The results showed that the progesterone exposure induced slight masculini- zation in female mosquitofish, influenced the oocyte maturation as revealed by histology of the ovaries, and caused severe damages to the liver and gills of adult female mosquitofish. > It also suppressed the mRNAs expression of vtg, er, cyp1a, and significantly enhanced the expression of ar mRNA in the liver.	Physiological responses and gene expression changes in the western mosquitofish (Gambusia affinis) exposed to progesterone at environmentally relevant concentrations	L. Hou et al. / Aquatic Toxicology 192 (2017) 69–77

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
69	Gambusia affinis	Androstenedione (AED)	0.04, 0.4, 4, 40, and 120 µg/L	180 d exposure, from embryo to adult	> the growth (length, body weight and condition factor) of fish was negatively correlated with AED concentration in females, but not in males. > The significant elongation of the ray and increment of segment numbers in the anal fin, were detected in all mosquitofish after exposure. >Moreover, AED exposure (0.4gµ/L) caused damages in gonads and reduced the number of pregnant females. These findings indicate that AED has adverse effects on the growth and development of the western mosquitofish after long-term exposure (180d). > Long-term exposure (180d) to AED, including environmentally relevant concentration (0.4 µg/L and 4 µg/L), induced masculinization in female mosquitofish under the experimental conditions.	Masculinization and reproductive effects in western mosquitofish (Gambusia affinis) after long-term exposure to androstenedione	L.-P. Hou et al. / Ecotoxicology and Environmental Safety 147 (2018) 509–515
70	zebrafish	17α,20β-dihydroxy-4-pregnen-3-one (DHP)	DHP 100 nM and RU486, 0.1 or 1 µM	exposure for 24 h	>In our present study, we first observed that fshb and lhb mRNA levels in the pituitary of male adult zebrafish were greatly inhibited by 3 weeks exposure to 10 nM estradiol (E2). >However, an additional 24 h 100 nM DHP exposure not only reversed the E2-induced inhibition, but also significantly increased the expression of fshb and lhb mRNA. > These stimulatory effects were also observed in male adult fish without E2 pretreatment, and a time course experiment showed that it took 24 h for fshb and 12 h for lhb to respond significantly. > Because these stimulatory activities were partially antagonized by a nuclear progesterone receptor (Pgr) antagonist mifepristone, we generated a Pgr-knockout (pgr–/–) model using the TALEN technique. With and without DHP in vivo treatment, fshb and lhb mRNA levels of pgr–/– were significantly lower than those of pgr+/+.	Progestin increases the expression of gonadotropins in pituitaries of male zebrafish	C WANG et al / Journal of Endocrinology (2016) 230, 143–156

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
71	juvenile sea bass	Drospirenone (DRO)	0.01 - 10 µg DRO/g	exposed through the diet for up to 31 days	>DRO was detected in the blood (4e27 ng/mL) of fish exposed to the highest concentration, with no significant bioaccumulation over time and no alteration of hepatic metabolizing enzymes, namely, CYP1A and CYP3A-catalysed activities and UDP-glucuronyltransferase (UGT). >Pregnenolone (P5), progesterone (P4), 17a-hydroxyprogesterone (17P4), 17a-hydroxypregnenolone (17P5), androstenedione (AD) and testosterone (T) were determined in plasma and gene expression of cyp17a1, cyp19a1a and cyp11b analysed by qRT-PCR in gonads. >The significant increase in plasmatic levels of 17P5, 17P4 and AD detected after 31 days exposure to 10 ng DRO/g together with the increased expression of cyp17a1 in females evidence the ability of DRO to alter steroid synthesis at low intake concentrations (7 ng DRO/day).	Drospirenone intake alters plasmatic steroid levels and cyp17a1 expression in gonads of juvenile sea bass	M. Blanco et al. / Environmental Pollution 213 (2016) 541e548
72 (see Serial No. 41)							
73	zebrafish	17a,20b-dihydroxy-4-pregnen-3-one (DHP),		function of DHP at early spermatogenetic stages	> results show that DHP treatment induced the proliferation of early spermatogonia, their differentiation into late spermatogonia and spermatocytes as well as expression of marker genes for these germ cell stages. > DHP-mediated stimulation of spermatogenesis and hence growth of spermatogenic cysts and the associated increase in Sertoli cell number may in part explain the elevated expression of Sertoli cell genes, but our data also suggest an up-regulation of the activity of the Igf signaling system.	A progestin (17α,20β-dihydroxy-4-pregnen-3-one) stimulates early stages of spermatogenesis in zebrafish	S.X. Chen et al. / General and Comparative Endocrinology 185 (2013) 1–9

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
74	Xenopus tropicalis	Levonorgestrel	0 - 1240 ng/L	exposure of mature females for 7 or 28 days	>The 28-day exposure caused reduced proportions of oocytes at immature, vitellogenic, and mature stages, and increased proportions of previtellogenic oocytes compared with the control. >The lowest tested concentration, 1.3 ng/L, increased the proportions of previtellogenic oocytes and reduced the proportions of vitellogenic oocytes, indicating inhibited vitellogenesis. > The present study shows that progestin concentrations found in the aquatic environment impaired oogenesis in adult frogs. Our results indicate that progestogenic effects on oocyte development include interrupted germ cell progression into meiosis and inhibited vitellogenesis.	Disrupted Oogenesis in the Frog Xenopus tropicalis after Exposure to Environmental Progestin Concentrations	SÄFHOLM ET AL. / BIOLOGY OF REPRODUCTION (2012) 86(4):126, 1–7
75	Fathead Minnow	amioda-rone, carbamazepine, clozapine, dexamethasone, fenofi-brate, ibuprofen, norethindrone, or verapamil.		OECD 210 ELS	>Exposure of FHM to carbamazepine, fenofibrate, and ibuprofen resulted in no significant adverse effects at the concentrations tested. >FHM survival was not impacted by verapamil exposure; however, growth was significantly decreased at 600 µg/L. >Dexamethasone-exposed FHM showed a significant decrease in survival at a concentration of 577 µg/L; however, growth was not impacted at the concentration tested. >Norethindrone exposure resulted in a significant decrease in survival and dry weight at 14.8 and 0.74 µg/L, respectively. >Exposure to amiodarone and clozapine resulted in a significant decrease in survival and a significant increase in growth at concentrations of 1020 and 30.8 µg/L, respectively.	Early Life-Stage Toxicity of Eight Pharmaceuticals to the Fathead Minnow, Pimephales promelas	Overturf et al / Arch Environ Contam Toxicol (2012) 62:455–464 DOI 10.1007/s00244-011-9723-6
76	Xenopus laevis	progesterone		Induction of mating and	> inducing ovulation in frogs which involved pre-treatment of frogs with salt solution followed by a	Induction of ovulation in Xenopus without hCG	Ogawa et al. / Reproductive

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
				ovulation by simply adding the natural maturation-inducing hormone into the water, in order to avoid injections.	mixture of estradiol and progesterone at high concentration. > The frequency and numbers of oocytes obtained were identical to those resulting from PMSG-hCG induction. > Fertilization rate of eggs ovulated by the new treatment method was comparable to eggs obtained by hCG-injection and juveniles developed normally.	injection: the effect of adding steroids into the aquatic environment	Biology and Endocrinology 2011, 9:11 http://www.rbej.com/content/9/1/11
77	fathead minnow, male	levonorgestrel	0 -100 ng/L	exposure for 14 d	>Males exposed to either 10 or 100 ng/L of levonorgestrel exhibited increased nest acquisition success and lower levels of aggression compared with control– control pairings, as well as decreases in multiple sperm motion characteristics.	Exposure to levonorgestrel increases nest acquisition success and decreases sperm motility in the male fathead minnow (Pimephales promelas)	Frankel et al / Environ Toxicol Chem. 2017 Dec 7. doi: 10.1002/etc.4054.
78	zebrafish, adult	norgestrel (NGT)	6.7 - 912 ng/L	exposure for 14 d	>The microarray analysis revealed that 11 545 transcripts were identified. Gene ontology analysis showed organ development, system development, multicellular organismal development, single-organism developmental process, and developmental process were significantly enriched. >A Venn diagram displayed 434 target genes involved in organ development, and these genes were common in these 5 development-related processes. Kyoto Encyclopedia of Genes and Genomes analysis showed that the notch signaling pathway was the top toxicity pathway, and it was selected as the target pathway for further qPCR analysis. >The qPCR analysis revealed significant and dose-dependent alterations of most target genes involved in the notch signaling pathway in the	Transcriptional and histological alterations in gonad of adult zebrafish after exposure to the synthetic progestin norgestrel.	Shi et al / Environ Toxicol Chem. 2017 Dec;36(12):3267-3276. doi: 10.1002/etc.3894.

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
					gonads, even at an environmentally relevant concentration of 6.7 ngL/L. The transcriptional patterns were consistent with the notch signaling cascade. >NGT significantly increased the frequency of mature sperm and decreased the frequency of immature sperm at all concentrations. Meanwhile, NGT treatment increased the percentage of mature vitellogenic oocytes and atretic follicles at 912 ng/L but decreased the percentage of immature vitellogenic oocytes.		
79		drospirenone (DRO), levonogestrel (LNG), progesterone (PRG)		liquid chromatographic-mass spectrometric method with solid-phase extraction, LOQ 0.03–0.11 ng/L	Analyzing freshwater samples from the Lake Balaton catchment area, we found influents where the concentration of these hormones was 0.26–4.30 (DRO), 0.85–3.40 (LNG), and 0.23–13.67 (PRG) ng/L. Out of 53 collecting places, 21 contained measurable progestin levels	HPLC-MS/MS analysis of steroid hormones in environmental water samples.	Avar et al./ Drug Test Anal. 2016 Jan;8(1):123-7. doi: 10.1002/dta.1829
80	zebrafish	17a,20b-dihydroxy-4-pregnen-3-one (17,20bP)	10 nM	exposure of solitary females or mixed pairs.	>exposure induced ovulation, but not spawning, in solitary females and both ovulation and spawning in male–female pairs. Transcription of the eicosanoid-synthesizing enzymes cytosolic phospholipase A2 (cPLA2) and COX-2 increased and LTC4 synthase decreased in peri-ovulatory ovaries of 17,20bP-exposed fish. >Ovarian PGF2a levels increased post-spawning in 17,20bP-exposed fish, but there was no difference in LTB4 or LTC4. Pre-exposure to cPLA2 or LOX inhibitors reduced 17,20bP-induced ovulation rates, while a COX inhibitor had no effect on ovulation or spawning. >Collectively, these findings suggest that	The role of eicosanoids in 17α, 20β-dihydroxy-4-pregnen-3-one-induced ovulation and spawning in Danio rerio.	Knight et al / Gen Comp Endocrinol. 2015 Mar 1;213:50-8. doi: 10.1016/j.ygcen.2014.12.014

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
					eicosanoids, in particular LOX metabolites, mediate 17,20bP-induced ovulation in zebrafish		
81	zebrafish	norgestrel	5 -100 ng/L	exposurefor 144 h post fertilization	>Norgestrel modulated the expression of Pgr and Vtg1 messenger (m)RNAs mainly at 96 hpf for all treatment groups. >In addition, norgestrel strongly altered the expression of Cyp11a1 mRNA above 5 ngL-1 (significant upregulation from 48 hpf to 120 hpf and significant downregulation for 144 hpf). >Norgestrel treatment could significantly induce expression of Cyp19a1a, Cyp11b, Gnrh2, Gnrh3, and Lhb mRNAs but inhibit transcripts of Hsd11b2 and Crh genes above 5 ngL-1 at different time points. >The transcriptional expression levels of Esr1, Ar, Star, Hsd17b3, Fshb, and Pomc were also mediated by 5 ngL-1 norgestrel or higher during different exposure periods.	A time-course transcriptional kinetics of the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal axes in zebrafish eleutheroembryos after exposure to norgestrel	Liang et al / Environ Toxicol Chem. 2015 Jan;34(1):112-9. doi: 10.1002/etc.2766
82	Eastern mosquitofish (Gambusia holbrooki), female			wild fish captured down stream of paper mill effluent	>Focused gene expression analyses of masculinized G. holbrooki from downstream of the Fenholloway River paper mill were indicative of androgen exposure, while genes related to reproduction indicated potential progesterone exposure. >Hepatic microarray analysis revealed an increase in the expression of metabolic genes in Fenholloway River fish, with similarities in genes and biological processes compared to G. holbrooki exposed to androgens. >Water samples collected downstream of the paper mill and at a reference site indicated that progesterone and androgen receptor active chemicals were present at both sites, which corroborates previous chemical analyses. Results indicate that G. holbrooki downstream of the	Exposure to paper mill effluent at a site in North Central Florida elicits molecular-level changes in gene expression indicative of progesterone and androgen exposure.	Brockmeier et al / PLoS One. 2014 Sep 8;9(9):e106644. doi: 10.1371/journal.pone.0106644

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
					Fenholloway River paper mill are impacted by a mixture of both androgens and progesterones.		
83	carp (Cyprinus carpio)	drospirenone (DRO), levonorgestrel (LNG), norethindrone (NOR) and cyproterone acetate (CPA)	0.01 - 500 µM	gonads from wild catches, in vitro exposure	>All tested progestogens significantly inhibited the synthesis of androgens: DRO (IC50: 3.8 µM) was the strongest inhibitor of CYP17 followed by CPA (IC50s: 183 µM). >Moreover, NOR (IC50: 0.4 µM), DRO (IC50: 1.8µM) and CPA (IC50s: 87 µM) inhibited CYP11b. An inhibition by NOR of ovarian CYP19 activity, and by DRO and CPA of 20b-HSD was also observed, but at rather high concentrations (500µM).	The in vitro interference of synthetic progestogens with carp steroidogenic enzymes	Fernandes et al / Aquat Toxicol. 2014 Oct;155:314-21. doi: 10.1016/j.aquatox. 2014.07.014
84	western mosquitofish (Gambusia affinis)	progesterone (P), testosterone (T) and 17β-estradiol (E2),	1 µg/L	exposure in mixtures or solitary for 8 d	>The results showed that expression patterns of Vtg subtype, ER subtype, AR subtype, MT and CYP1A genes in male mosquitofish varied according to tissue and specific hormone stress. >Vtg subtype mRNA expression was induced in the liver in E2-added treatments, and an up-regulation of ERα mRNA expression was also observed. In addition, hormone treatments increased three Vtg subtype mRNA expression levels in the testis, at least to some extent. > All hormone treatments significantly inhibited ERα, ERβ and ARβ mRNA expression in the testis. Some of hormone treatments could affect MT and CYP1A gene expression in mosquitofish. >In general, multiple hormone treatments showed different effects on target gene expression compared with corresponding hormone alone.	Effects of steroid hormones on reproduction- and detoxification-related gene expression in adult male mosquitofish, Gambusia affinis	Huang et al / Comp Biochem Physiol C Toxicol Pharmacol. 2013 Jun;158(1):36-43. doi: 10.1016/j.cbpc.201 3.05.001
85	Fathead minnow	progesterone (P4)	10-8 and 10-6 M	testis explants were treated in vitro, exposure for 6 and 12 h	> P4 significantly increased testosterone (T) production in the FHM testis but did not affect 11-ketotestosterone. > Gene network analysis revealed that insulin growth factor (Igf1) and tumor necrosis factor	Transcriptomic profiling of progesterone in the male fathead minnow (Pimephales promelas) testis	Chisti et al / Gen Comp Endocrinol. 2013 Oct 1;192:115-25. doi:

Serial No.	Organism/ Test species/ Age	Substance tested	Concentration range	Experimental approach	Results / Conclusions	Title	Reference
					receptor (Tnfr) signaling was significantly depressed with P4 treatment after 12 h. >There was also a 20% increase in a gene network for follicle-stimulating hormone secretion and an 18% decrease in genes involved in vasopressin signaling. > Genes in steroid metabolism (e.g. star, cyp19a, 11bhsd) were not significantly affected by P4 treatments in this study, and it is hypothesized that pre-existing molecular machinery may be more involved in the increased production of T rather than the de novo expression of steroid-related transcripts and receptors. >There was a significant decrease in prostaglandin E synthase 3b (cytosolic) (ptges3b) after treatment with P4, suggesting that there is cross talk between P4 and prostaglandin pathways in the reproductive testis.		10.1016/j.ygcen.2013.04.033.

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D.3 Results of the literature research for glucocorticoids

Table 250: Results of the literature research for glucocorticoids

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
1	Mammalian cell lines transfected with trout corticosteroid receptors (GR1, GR2, and MR)	hydrocortisone, prednisolone, betamethasone, beclomethasone, fluticasone, budesonide, momethasone, clobetasone, methylprednisolone, dexamethasone	nM	*in vitro*	> significantly higher activity with GR2 than with GR1 >EC50 values of Tested GCs:for GR2 range 0.386 - 1.86 nM, EC50 values for GR1 range 40.98-61.09 nM > Overall, there is approximately an 8-12-fold difference in the sensitivity (as efficacy) of GCs between GR2 and GR1. > None of the tested GCs produced significant activity with fish MR.	Synthetic Glucocorticoids in the Environment: First Results on Their Potential Impacts on Fish	Kugathas et al. dx.doi.org/10.1021/es104105e | Environ. Sci. Technol. 2011, 45, 2377–2383
1	adult fathead minnows	prednisolone, beclomethasone dipropionate	1 µg/L	exposure for 21 days	> Plasma glucose concentrations were increased and leucocytes were reduced significantly in GC-exposed groups compared to the control group. The effect of beclomethasone was higher than that of prednisolone, but the difference was not significant. > The anti-inflammatory properties of GCs were also revealed, as the total leucocyte count was reduced significantly by both prednisolone and beclomethasone. Total leucocytes were reduced significantly in GC-exposed groups for prednisolone and beclomethasone, respectively) compared to the control group > There were no differences in the condition factor, liver-somatic index, or gonadosomatic index between control and treated groups	Synthetic Glucocorticoids in the Environment: First Results on Their Potential Impacts on Fish	Kugathas et al. dx.doi.org/10.1021/es104105e | Environ. Sci. Technol. 2011, 45, 2377–2383

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
1	adult fathead minnows	Beclomethasone dipropionate	100 ng/L, 1 µg/L, and 10 µg/L	exposure for 21 days	> dose-related increase of plasma glucose levels in beclomethasone dipropionate - treated fish. Glucose levels in fish exposed to 1 and 10 µg/L were significantly higher than that of control fish	Synthetic Glucocorticoids in the Environment: First Results on Their Potential Impacts on Fish	Kugathas et al. dx.doi.org/10.1021/es104105e | Environ. Sci. Technol. 2011, 45, 2377–2383
2	adult male fathead minnow	beclomethasone dipropionate	10, 100 and 1000ng/L	21-day exposure, with oscillating exposure (Exp 1) and constant exposure (Exp.2)	> The comparison of measured plasma concentrations of BDP + 17-BMP in fish from Experiment 1 and Experiment 2 sampled from water containing similar concentrations of BDP (approx. 10 ng/L) confirmed that drug plasma concentrations were determined by BDP water concentrations at the sampling time. > Blood glucose was elevated after oscillating exposure to 100 ng BDP/L or higher in a concentration-dependent manner > GR expression in the liver was significantly induced by 3.2-fold after oscillating exposure to 100 and 1000 ng BDP/L in Experiment 1; These results were confirmed in Experiment 2, in which sustained exposure to 1000, but not to 10, ng BDP/L significantly induced GR by 2.2-fold > In Exp. 1 the percentage of lymphocytes was significantly reduced after oscillating exposure to 1000 ng BDP/L for 21-days; in Experiment 2, effect concentration was significantly reduced compared to Experiment 1, with lymphocyte count decreased by 64 ± 13% after exposure to 10 ng BDP/L > Exposure to BDP caused the induction of male SSCs in both experiments, confirming the androgenic effects of BDP	Internal exposure dynamics drive the Adverse Outcome Pathways of synthetic glucocorticoids in fish	Margiotta-Casaluci, L. et al. (2016). "Internal exposure dynamics drive the Adverse Outcome Pathways of synthetic glucocorticoids in fish." Scientific Reports 6: 21978.

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
3	bluegill, *Lepomis macrochirus*			glucocorticoids levels in newly captured fish; circadian cycle; stress responsiveness of glucocorticoids	> baseline glucose concentration did not vary on a diel basis, whereas baseline cortisol concentration did. > Maximum and stress-induced glucose responsiveness varied significantly among several time periods with lowest values recorded at midnight and higher values at mid-day > Maximum and stress-induced cortisol responsiveness were consistent across time periods. >there is apparently an absence of strong GC diel patterns	Diel patterns of baseline glucocorticoids and stress responsiveness in a teleost fish (bluegill, Lepomis macrochirus)	A. Cousineau Can. J. Zool. 92: 417–421 (2014) dx.doi.org/10.1139 /cjz-2014-0054
4	Largemouth bass *Micropterus salmoides*, brown bullhead *Ameiurus nebulosus*, white sucker *Catostomus commersonii*, pumpkinseed *Lepomis gibbosus*, and logperch *Percina caprodes*	Field sampling of fish			>largemouth bass possessed altered baseline GC concentrations and brown bullhead and logperch had altered GC responses to a stressor. >White sucker and pumpkinseed did not demonstrate any alteration in baseline or post-stress GC concentrations. > results show that different species residing in identical habitats can demonstrate a variety of responses to environmental stress, highlighting the variation in physiological ability to cope under poor environmental conditions, as well as the difficulty of predicting GC dynamics inwild animals.	Stress in the neighborhood: Tissue glucocorticoids relative to stream quality for five species of fish	King et al. Science of the Total Environment 547 (2016) 87–94
5	male *Hoplias malabaricus*	diclofenac and dexamethasone	diclofenac (0; 0.2 ;2.0 or 20.0 µg/kg) or dexame-thasone	trophic exposure	> Trophicexposureof H. malabaricus to dexamethasone caused an increase in antioxidant system (GPx,CAT,GST,andGSH) and LPO in liver > it reduced antioxidant system (GPX and GST activities and GSH) in gonads. > Both diclofenac and dexamethasone reduced the	Effects oftrophic exposure to dexamethasone and diclofenac in freshwater fish	I.C. Guiloskietal./ Ecotoxicology and Environmental Safety 114 (2015) 204–211

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
			(0;0.03;0.3o r3.0 µg/kg)		levels of testosterone, causing impairment to reproduction.		
6	common carp (*Cyprinus carpio)*	clobetasol propionate (CP), clobetasone butyrate (CB)	1 µg/L	exposure individually or in mixture for 21 days.	> Bioconcentration factor (BCF) of CB was calculated as 100, and BCF of CP was less than 16. > No effects were found in fish erythrocyte and leukocyte numbers and serum glucose levels after exposure to the selected GCs. > serum concentrations of free amino acids significantly increased in GC-exposed groups. > exposures to synthetic GCs at relatively low concentrations seemed to cause enhancement of protein degradation and subsequent increase of serum free amino acids without a corresponding increase in serum glucose levels, an effect which might be related to partial induction of gluconeogenesis by GC	Uptake and biological effects of synthetic glucocorticoids in common carp (Cyprinus carpio)	K. Nakayama et al. / Marine Pollution Bulletin 85 (2014) 370–375
7	transgenic SR4G zebrafish reporter line				> development of SR4G zebrafish reporter line with six glucocorticoid response elements used to promote expression of a short half-life green fluorescent protein following glucocorticoid receptor activation The green fluorescent protein > expression in response to transgene activation was high in a variety of tissues including the brain, and provided single-cell resolution in the effected regions. The specificity of these responses is demonstrated using the partial agonist mifepristone and mutation of the glucocorticoid receptor. > the reporter line also modeled the temporal dynamics of endogenous stress response signaling, including the increased production of the glucocorticoid cortisol following	A transgenic zebrafish model for monitoring glucocorticoid receptor activity	Krug II et al 2014; Genes, Brain and Behavior (2014) 13: 478–487

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
					hyperosmotic stress and the fluctuations of basal cortisol concentrations with the circadian rhythm.		
8					Review on the current state of knowledge on egg cortisol in fishes, and the relationships linking maternal stress/state to egg cortisol and offspring phenotype/fitness	Glucocorticoids in fish eggs: causes of variation and effects on offspring phenotype	Sopinka et al. (2017)
9	zebrafish, embryo			whole embryo cortisol (WEC) and corticosteroid-related genes were measured in embryos from 6 to 120 h post fertilisation (hpf) by enzyme linked immunosorbent assay (ELISA) and quantitative real-time polymerase chain reaction (qRT-PCR).	> WEC levels demonstrated a biphasic pattern during development with a decrease from 0 to 36 hpf followed by a progressive increase towards 120 hpf; accompanied by a significant and sustained increase in the expression of genes encoding cyp11b1 (GC biosynthesis), hsd11b2 (GC metabolism) and gr (GC receptor) from 48 to 120 hpf. > Metyrapone (Met), an inhibitor of 11β-hydroxylase (encoded by cyp11b1), and cyp11b1 morpholino (Mo) knockdown significantly reduced basal and stress-induced WEC levels at 72 and 120 hpf but not at 24 hpf. > Spontaneous hatching and swim activity were significantly affected by manipulation of GC action from approximately 48 hpf onwards	Physiological roles of glucocorticoids during early embryonic development of the zebrafish (Danio rerio)	K. S. Wilson et al; J Physiol 591.24 (2013) pp 6209–6220
10	zebrafish, fathead minnow, medaka, roach, stickleback, carp	natural estrone, estradiol, estriol and synthetic 17α-ethinylestradiol (EE2), diethylstilbestrol (DES)	2 and 10 ng/L	> in vitro ERα activation assays > in vivo: exposure for 7 days; responsiveness for hepatic vitellogenin (VTG) mRNA induction	> zebrafish ERα was found to be the most responsive and carp and stickleback ERα the least responsive to natural steroid estrogens > exposure to EE2 with an ERα-mediated response sensitivity order of zebrafish>medaka > roach > fathead minnow > carp > stickleback > For VTG mRNA induction in vivo, the order of species responsiveness was: rainbow trout (not tested in the ERα activation assays) > zebrafish > fathead minnow > medaka > roach > stickleback > carp. > responses to steroid estrogens in vitro via ERα	Comparative responsiveness to natural and synthetic estrogens of fish species commonly used in the laboratory and field monitoring	A. Lange et al. / Aquatic Toxicology 109 (2012) 250–258

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
					compared well with those seen in vivo (VTG induction for exposure to EE2) showing in vitro screening of chemicals using fish ERα-mediated responses indicative of estrogenic responses (VTG induction) in vivo		
11				modelling approach to predict the total environmental concentration of all synthetic GCs	> LF2000-WQX model predicts mean concentrations up to 30 ng/L of total GCs in surface water as a best-case scenario when the lowest excretion and highest removal rates in sewage treatment works > mean concentrations up to 850 ng/L were predicted when the highest excretion and lowest removal rates are considered.	Prediction of environmental concentrations of glucocorticoids: The River Thames, UK, as an example	S. Kugathas et al. / Environment International 40 (2012) 15–23
12	zebrafish	dexamethasone, prednisolone, triamcinolone	50 pM - 50 nM	>exposure of embryo for 120 h, of adults for 4 days >expression of eleven glucocorticoid-responsive genes in zebrafish larvae and liver of adult male zebrafish	> expression of pepck, baiap2 and pxr was up-regulated in zebrafish larvae and the expression of baiap2, pxr and mmp-2 was up-regulated in adult zebrafish exposed to glucocorticoids at concentrations equivalent to total glucocorticoids reported in environmental samples. > responsiveness of the specific genes were sufficiently robust in zebrafish larvae exposed to a complex environmental sample detected with in vitro glucocorticoid activity equivalent to 478pM dexamethasone(DEX-EQ) and confirmed to contain low concentration (0.2ng/L or less) of the targeted glucocorticoids, and possibly other glucocorticoid-active compounds	Glucocorticoid activity detected by in vivo zebrafish assay and in vitro glucocorticoid receptor bioassay at environmental relevant concentrations	Q. Chen et al./Chemosphere144(2016)1162–1169
13				determination of nine glucocorticoids in river waters and sewage.	> The method obtains LODs for glucocorticoids at low ng/L levels in aqueous environmental matrices (0.5–20 ng/L depending on the matrix and the analyte) > Cortisone, cortisol, prednisone and prednisolone were frequently determined in influent sewage samples between 21 and 285 ng/L	Determination of glucocorticoids in sewage and river waters by ultra-high performance liquid chromatography–tandem mass spectrometry	P. Herrero et al. / J. Chromatogr. A 1224 (2012) 19– 26

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
14				occurrence androgens, estrogens, glucocorticoids and progestagens were investigated in two different types of wastewater treatment plant	> glucocorticoids (171–192 ng L−1 in influent, 2.2–6.3 ng L−1 in effluent, N.D.–4.4 ng g−1 in dewatered sludge) > progestagens (39.6–40.5 ng L−1 in influent, 6.9–12.1 ng L−1 in effluent, N.D. in dewatered sludge)	Occurrence and fate of androgens, estrogens, glucocorticoids and progestagens in two different types of municipal wastewater treatment plants	Liu et al 2012 / J. Environ. Monit., 2012, 14, 482-491, DOI: 10.1039/C1EM10783F
15				28 steroids were investigated in a typical swine farm with RRLC-MS/MS with lagoon waste disposal systems, in south China	> Nineteen, 22 and 8 of 28 steroids were detected at concentrations ranging from 2.2 ± 0.1 ng/g (androsta-1,4-diene-3,17-dione) to 14,400 ± 394 ng/g (progesterone) in the feces samples, from 6.1 ± 2.3 ng/L (17β-boldenone) to 10,800 ± 3190 ng/L (norgestrel) in the flush water samples, and from 5.0 ± 0.2 ng/g (progesterone) to 225 ± 79.4 ng/g (5α-dihydrotestosterone) in the suspended particles	Steroids in a typical swine farm and their release into the environment	Liu et al 2012 / water research 46 (2012) 3754e3768
16	fathead minnow	dexamethasone	500 µg /L	21-d reproduction and 29-d embryo–larvae assays	> Exposure to 500 µg dexamethasone/L in the 21-d test caused reductions in fathead minnow fecundity and female plasma estradiol concentrations and increased the occurrence of abnormally hatched fry. > Female fish exposed to 500 µg dexamethasone/L also displayed a significant increase in plasma vitellogenin protein levels, possibly because of decreased spawning. > A decrease in vitellogenin messenger ribonucleic acid (mRNA) expression in liver tissue from females exposed to the high dexamethasone concentration lends support to this hypothesis. > Histological results indicate that a 29-d embryo–larval exposure to 500 µg dexamethasone/L caused	Effects of a glucocorticoid receptor agonist, dexamethasone, on fathead minnow reproduction, growth, and development	C.A. LaLone et al./ Environ. Toxicol. Chem. 31, 2012

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
					a significant increase in deformed gill opercula. > Fry exposed to 500 µg dexamethasone/L for 29 d also exhibited a significant reduction in weight and length compared with control fry.		
17	in vitro assays	Dexamethasone served as the reference compound		REVIEW on suitability of in vitro assays to detect endocrine activity in water	>Five in vitro glucocorticoid assays covering two assay types, yeast reporter gene assays and mammalian reporter gene assays > The yeast glucocorticoid assay was almost four orders of magnitude less sensitive than themammalian reporter gene assays, with a calculated method detection limit (MDL) of 1,400,000 ng/L. >Four mammalian reporter gene assays were considered, including GRCALUX, GR-GeneBLAzer, HG5LN-Gal4-GR and TGRM-luc. >Re-evaluated Dexa concentration-effect curves revealed that GR-GeneBLAzer was the most sensitive, with a MDL of 34 ng/L, followed by GR-CALUX, with a MDL of 45 ng/L. TGRM-luc and HG5LNGal4-GR were less sensitive, with MDLs of 180 and 640 ng/L, respectively.	Analysis of the sensitivity of in vitro bioassays for androgenic, progestagenic, glucocorticoid, thyroid and estrogenic activity: Suitability for drinking and environmental waters	F.D.L. Leusch et al. / Environment International 99 (2017) 120–130
18		surface water, wastewater and sludge samples in China		5 glucocorticoids (cortisol, cortisone, prednisone, prednisolone, dexamethasone) in surface water, wastewater and sludge samples.	>No glucocorticoid was detected in all sludge and effluent samples. > Wastewater influent concentration of Glucocorticoids were in the range of 5-45 ng/L	Trace analysis of 28 steroids in surface water, wastewater and sludge samples by rapid resolution liquid chromatography–electrospray ionization tandem mass spectrometry	S. Liu et al. / J. Chromatogr. A 1218 (2011) 1367–1378
19	*Cyprinus carpio*	synthetic and natural progestogens and		effects on leucocytes	>Injection of cortisol led to an increased spleeno-somatic index (IS) on day 21 post-injection (pi) and immunosuppressive effects measured as decreased	In vivo treatment with progestogens causes immunosuppression of	C. PIETSCH ET AL. / Journal of Fish

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
		the glucocorticoid cortisol		isolated from their kidneys	nitric oxide (NO) production and increased arginase activity in isolated leucocytes on days 14 and 21 pi, respectively. >reduced NO production was also observed after injection of the synthetic progestogens, levonorgestrel (LEV) and medroxyprogesterone acetate. >In addition, LEV influenced arginase activity in head kidney cells on day 14 and day 21 pi.	carp Cyprinus carpio leucocytes by affecting nitric oxide production and arginase activity	Biology 2011, 79, 53–69
20	zebrafish	Clobetasol propionate	≤1000 nM CP	Zebrafish embryos were exposed to ≤1000 nM from ~1 h post fertilization (hpf) to 96 hpf, and clobetasol propionate (CP) uptake, survival after bacterial lipopolysaccharide (LPS) challenge, and expression of inflammation-related genes were examined.	> CP causes significant immunosuppression in zebrafish embryos already at 0.1 nM. > The internal CP concentration was quantifiable after exposure to ≥10 nM CP for 96 h > The bioconcentration factor (BCF) of CP was determined to be between 16 and 33 in zebrafish embryos. > CP-exposed embryos showed a significantly higher survival rate in the LPS challenge assay after exposure to ≥0.1 nM in a dose dependent manner. This effect is an indication of immunosuppression. > the regulation pattern of several genes related to LPS challenge in mammals supported our results, providing evidence that LPS-mediated inflammatory pathways are conserved from mammals to teleost fish. >Anxa1b, a GC-action related anti-inflammatory gene, was significantly down-regulated after exposure to ≥0.05 nM CP.	Clobetasol propionate causes immunosuppression in zebrafish (Danio rerio) at environmentally relevant concentrations	Hidasi et al./ Ecotoxicology and Environmental Safety 138 (2017) 16–24
21	Eurasian perch juveniles	Cortisol, 11-deoxycorticosterone (DOC)	Cortisol and DOC were injected at 0.8 mg kg−1 and 0.08 mg	corticosteroid injections on the short term (from 1 to 72 h), sampling after 1, 6, 24 and 72 h after injection	> Cortisol increased plasma lysozyme activity 72 h post-injection, C-type lysozyme expression in spleen from 1 to 72 h post-injection, and favoured blood neutrophils at the expense of a mixture of lymphocytes and thrombocytes. >Moreover, 6 h after injection, cortisol reduced	First evidence of the possible implication of the 11-deoxycorticosterone (DOC) in immune activity of Eurasian	C. Mathieu et al. / Comparative Biochemistry and Physiology, Part A 165 (2013) 149–158

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
			kg−1 body weight		expression levels of the pro-inflammatory cytokine TNF-α in spleen. > DOC had no effects on the immune variables measured in plasma, but increased expression levels of C-type lysozyme and apolipoprotein A1 mRNA in both gills and spleen. > DOC stimulated its putative signalling pathway by increasing expression of mineralocorticoid receptor and 11β-hydroxysteroid dehydrogenase-2 in spleen.	perch (Perca fluviatilis, L.): Comparison with cortisol	
22	common carp (*Cyprinus carpio*)	glucocorticoids present in sewage treatment plant (STP) effluent clobetasol propionate and betamethasone 17-valerate		responses to bacterial infection (Aeromonas veronii) of common carp exposed to clobetasol propionate	>Clobetasol propionate exposure did not affect bacterial infection-associated mortality. > In fish infected with A. veronii but not exposed to clobetasol propionate, head kidney weight and number of leukocytes in the head kidney were significantly increased ($p<0.05$), whereas these effects were not observed in infected fish exposed to clobetasol. >This suggests that clobetasol propionate alleviated bacterial infection-associated inflammation	Occurrence of glucocorticoids discharged from a sewage treatment plant in Japan and the effects of clobetasol propionate exposure on the immune responses of common carp (Cyprinus carpio) to bacterial infection	NAKAYAMA, et al. / Environ Toxicol Chem 35, 2016
23	Rainbow trout hepatocytes	Cortisol	100 ng/mL	Hepatocytes were treated with lipopolysaccharide (LPS) for 24 h either in the presence or absence of cortisol.	>LPS stimulated heat shock protein 70 expression, enhanced glycolytic capacity, and reduced glucose output. >LPS stimulated mRNA abundance of cytokines and serum amyloid protein A (SAA), while suppressors of cytokine signaling (SOCS)-3 was reduced. > Cortisol increased mRNA abundances of IL-1b, SOCS-1 and SOCS-2, while inhibiting either basal or LPS-stimulated IL-8, TNF a2 and SAA. These cortisol-mediated effects were rescued by Mifepristone, a glucocorticoid receptor antagonist. >Altogether, cortisol modulates the molecular immune response in trout hepatocytes.	Cortisol modulates the expression of cytokines and suppressors of cytokine signaling (SOCS) in rainbow trout hepatocytes	A.M. Philip et al. / Developmental and Comparative Immunology 38 (2012) 360–367

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24				REVIEW zebrafish as a model system for glucocorticoid receptor research	>Both the zebrafish and the human genome contain only a single gene encoding the GR. In all other fish species studied thus far, two GR genes have been found. >The zebrafish contains a C-terminal GR splice variant with high similarity to the human GRβ, which has been shown to be a dominant-negative inhibitor of the canonical GRα and may be involved in glucocorticoid resistance. >Thus, zebrafish embryos are potentially a useful model system for glucocorticoid receptor research, but currently only a limited number of tools is available.	The zebrafish as a model system for glucocorticoid receptor research	Schaaf et al / Steroids Volume 153, Issue 1, May 2009, Pages 75-82
25 / 60	zebrafish			REVIEW	>Zebrafish are easily genetically modified, so the expression of GRβ can be varied, creating an in vivo model for GRβ-induced glucocorticoid resistance. > two types of in vivo models for studying glucocorticoid resistance, that will be used to study the molecular mechanism of glucocorticoid signaling and resistance	The zebrafish as an in vivo model system for glucocorticoid resistance	Schoonheim et al. / Steroides Volume 75, Issue 12, December 2010, Pages 918-925
26				assessment on the presence and extent of glucocorticogenic activity in Dutch surface waters	> results show glucocorticogenic activity in the range of <LOD – 2.4 ng dexamethasone equivalents L-1 (dex EQs) in four out of eight surface waters. >An exploratory time-series study to obtain a more complete picture of the yearly average of fluctuating glucocorticogenic activities at two sample locations demonstrated glucocorticogenic activities ranging between <LOD – 2.7 ng dex EQs L-1	Occurrence of glucocorticogenic activity in various surface waters in The Netherlands	M. Schriks et al. /Chemosphere 93 (2013) 450–454
27	zebrafish larvae	dexamethasone (DEX)		caudal fin amputation tissue repair capacity at later life stages (5, 14, and 24 days	> prior exposure to DEX significantly delays but does not prevent wound healing at all life stages studied. > DEX-induced impairments on wound healing were fully restored to normal levels with longer post amputation recovery	Long-lasting effects of dexamethasone on immune cells and wound healing in the zebrafish	Sharif et al. 2015 DOI:10.1111/wrr.1 2366

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				post fertilization [dpf]) in zebrafish larvae	time. > Further analyses revealed that DEX mainly exerted its detrimental effects in the early phase (0–5 hours) of wound-healing process. Specifically, we observed the following events: (1) massive amount of cell death both by necrosis and apoptosis; (2) significant reduction in the number as well as misplacement of macrophages at the wound site; (3) aberrant migration and misplacement of neutrophils and macrophages at the wound site. > These events were accompanied by significant changes in the expression of genes involved in tissue patterning, including up-regulation of FKBP5 6 hours post DEX exposure and that of Wnt3a and RARc at 24 hours post amputation. > evidence that DEX exposure during early sensitive periods of development appears to cause permanent alterations in the cellular/molecular immune processes that are involved in the early phase of wound healing in zebrafish.		
28	GR-CALUX bioassay—a human osteosarcoma cell line stably co-transfected with human GR-regulated luciferase gene constructs	lobetasol propionate, clobetasone butyrate, betamethasone 17-valerate ,difluprednate, betamethasone17,21-dipropionate, Dex , betamethasone , 6α-methylprednisolone		GC-Responsive Chemical-ActivatedLUciferase gene eXpression (GR-CALUX)	>tested GCs demonstrateddose-dependent agonistic effects in the GR-CALUXassay and theirEC50 valueswere calculated for estimation of relative potencies (REPs) compared toDex. > The GR agonistic potency was inthe rank of: clobetasol propionate > clobetasone butyrate > betamethasone 17-valerate > difluprednate > betamethasone17,21-dipropionate > Dex > betamethasone > 6α-methylprednisolone > prednisolone > cortisol. >The GR agonistic activity in STP effluents asmeasured inDex-equivalent (Dex-EQ) activities ranged from <3.0–78ng L−1	Detection of glucocorticoid receptor agonists in effluents from sewage treatment plants in Japan	G. Suzuki et al. / Science of the Total Environment 527–528 (2015) 328–334

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
		,prednisolone , cortisol					
29	zebrafish			development of ELISA protocol	> optimized sample collection as well as cortisol extraction methods and developed a home-made ELISA protocol for measuring whole-body cortisol level in zebrafish larvae. > measurement of cortisol values in whole larval homogenates > diverse environmental stimuli can lead to a dose-dependent cortisol increase in larval zebrafish	An Optimized Whole-Body Cortisol Quantification Method for Assessing Stress Levels in Larval Zebrafish	Yeh C-M, Glöck M, Ryu S (2013) PLoS ONE 8(11): e79406. doi:10.1371/journal.pone.0079406
30	Atlantic salmon (Salmo salar)	50 µg cortisol g-1 body weight in an implant based on vegetable lipids		45 days chronically elevated levels of cortisol on the immune response and susceptibility to experimental infection with infectious pancreatic necrosis virus (IPNV)	> Cortisol implantation compared with sham-implanted fish had increased levels of plasma cortisol at 45 DAI. > The relative expression of Interferon alpha-1 (IFNa-1), Myxo virus-1 Mx, Heat-shock protein 70 (HSP70), Serum amyloid A (SAA), Glucocorticoid receptor (GR) and Heat-shock protein 90 (HSP90) tends to be down-regulated by cortisol implantation. > higher prevalence of fish with detectable levels of IPNV, as measured by cell culture and RT-PCR, in the cortisol-implanted group challenged with IPNV (0 ¼ 0.0305) relative to the group that received a sham implantation. > cortisol seems to delay the induction of the antiviral IFNa-1 pathway and Mx mRNA expression.	Slow release cortisol implants result in impaired innate immune responses and higher infection prevalence following experimental challenge with infectious pancreatic necrosis virus in Atlantic salmon (Salmo salar) parr	K. Gadan et al. / Fish & Shellfish Immunology 32 (2012) 637e644
31		Betamethasone dipropionate, Betamethasone valerate, Clobetasone butyrate,		sewage treatment plant effluent in Japan	>Cortisol was detected in more than half of (27 out of 50) analyzed effluent samples at concentrations in the range of ND-1.36 ng/L, indicating continu-ous discharge of natural GC via STP effluent. > On the other hand, dexamethasone+betamethasone, prednisolone,	Determination of natural and synthetic glucocorticoids in effluent of sewage treatment plants using ultrahigh performance	Isobe et al / Environ Sci Pollut Res (2015) 22:14127–14135

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		Difluprednate, Clobetasol propionate, Cortisol, Dexamethasone, Betamethasone, Methylprednisolone, Prednisolone, Beclomethasone			betamethasone valerate, and clobetasol propionate were detected in 25, 8, 20, and 9 samples among 50 effluent sam- ples, respectively, suggesting not extreme but significant administration of synthetic GCs.	liquid chromatography-tandem mass spectrometry	
32				REVIEW	> summarizes a number of studies that have examined changes in the expression of genes encoding proteins critical for the functioning of the HPI axis, and the associated cortisol response in developing zebrafish embryos and larvae.	Molecular programming of the corticosteroid stress axis during zebrafish development	Alsop et al. / Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology Volume 153, Issue 1, May 2009, Pages 49-54 https://doi.org/10.1016/j.cbpa.2008.12.008
33	Senegalese sole	dexamethasone (DXM)	0.1, 1 and 10 ppm	exposure of post-metamorphic individuals (21 days after hatching)	>Short-term exposure to 0.1, 1 and 10 ppm DXM provoked a reduction of pomcb transcripts and an increase of crfbp mRNAs in a dose-dependent manner at 48 and 72 h after treatment. >Moreover, g-type lysozyme transcript levels decreased significantly at 72 h whereas hamp1 mRNA levels increased at 48 h after exposure. >Long-term DXM treatment (10 ppm DXM) affected negatively weight of soles (w20% lower than controls). >Moreover, reduced mRNA levels were observed for pomcb after 1 week and igf-I and hamp1 after 2 weeks. > In contrast, crfbp and crf increased mRNA levels	Dexamethasone modulates expression of genes involved in the innate immune system, growth and stress and increases susceptibility to bacterial disease in Senegalese sole (Solea senegalensis Kaup, 1858)	E. Salas-Leiton et al. / Fish & Shellfish Immunology 32 (2012) 769e778

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
					after 2 weeks. hsp70 exhibited a dual response increasing transcript levels at 1 week after treatment and reducing thereafter. > No significant changes in gene expression were observed at any time during this study for tgb, trh, hsp90aa, pomca, gr1 and gr2. > Finally, a challenge experiment using the pathogen Photobacterium damselae subsp piscicida confirmed earlier and higher mortalities in DXM-treated animals.		
34	goldfish	dexamethasone, cortisol	1 µg/g body weight; 0.1–10 µM	role of glucocorticoids as modulators of clock genes expression in the liver of goldfish.	> In fish maintained under a 12L:12D photoperiod, an intraperitoneal injection at Zeitgeber Time 2 of dexamethasone (1 µg/g body weight) induced per1 genes while decreased gbmal1a and gclock1a expression in the liver at 8 h post-injection. > A 4-h in vitro exposure of goldfish liver to cortisol (0.1–10 µM) also induced gper1 genes in a concentration-dependent manner and produced a concentration-dependent induction of gper1 genes. > glucocorticoid analog led to a decrease in gbmal1a and gclock1a transcripts, while the other clock genes analyzed were unaffected. > The induction of gper1a and gper1b by dexamethasone in vitro was observed at short times (2 h), whereas the reductions of gbmal1a and gclock1a transcripts needed longer exposure times (8 h) to the glucocorticoid to be significant.	Performing a hepatic timing signal: glucocorticoids induce gper1a and gper1b expression and repress gclock1a and gbmal1a in the liver of goldfish	Sánchez-Bretaño et al. /J Comp Physiol B (2016) 186:73–82, DOI 10.1007/s00360-015-0936-2
35	zebrafish			>Danio rerio, cortisone can be further reduced to 20β-hydroxycortisone. This reaction is	>We found that hsd11b2 and hsd20b2 transcripts were up-regulated upon cortisol treatment. > a cortisol-independent, short-term physical stressor led to the up-regulation of hsd11b2 and hsd20b2 along with several HPI axis genes. >The morpholino-induced knock down of hsd20b2	Zebrafish 20β-Hydroxysteroid Dehydrogenase Type 2 Is Important for Glucocorticoid	Tokarz et al / PLoS ONE 8(1): e54851. doi:10.1371/journal.pone.0054851

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				catalyzed by 20β-HSD type 2 >20β-HSD type 2 is involved in cortisol catabolism and stress response	in zebrafish embryos revealed no developmental phenotype under normal culture conditions, but prominent effects were observed after a cortisol challenge. >Reporter gene experiments demonstrated that 20β-hydroxycortisone was not a physiological ligand for the zebrafish glucocorticoid or mineralocorticoid receptor but was excreted into the fish holding water. > 20β-HSD type 2, together with 11β-HSD type 2, represents a short pathway in zebrafish to rapidly inactivate and excrete cortisol.	Catabolism in Stress Response	
36	Fathead Minnows	beclomethasone dipropionate	0.1, 1 and 10 µg/L	>exposure for 21 days	>concentration-related increase in plasma glucose concentration and a decrease in blood lymphocyte count. >Induction of male secondary sexual characters and a decreasing trend in plasma vitellogenin (Vtg) concentrations in female fish were observed with increasing exposure concentration of BCMD. > Expression profiles of selected genes (phosphoenolpyruvate carboxykinase - PEPCK, glucocorticoid receptor - GR, and Vtg) in liver also demonstrated concentration-related effects at all three tested concentrations. > The results suggest that GCs could cause effects in lower micrograms per liter concentrations that could be environmentally relevant for total GCs present in the environment.	Metabolic and Reproductive Effects of Relatively Low Concentrations of Beclomethasone Dipropionate, a Synthetic Glucocorticoid, on Fathead Minnows	Kugathas et al. / Environ. Sci. Technol., 2013, 47 (16), pp 9487–9495 DOI: 10.1021/es401933 2
37				Creation of the Glucocorticoid Responsive In vivo Zebrafish Luciferase activitY	The GRIZLY assay detects stress-induced glucocorticoid production in single zebrafish larvae, measures disruption of glucocorticoid signaling by an organotin pollutant metabolite, and specifically	A Chemical Screening System for Glucocorticoid Stress Hormone Signaling in an Intact Vertebrate	Weger et al. /ACS Chemical Biology 2012 7 (7), 1178-1183 DOI:

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				assay to monitor glucocorticoid signaling in vivo	identifies a compound stimulating endogenous glucocorticoid production in a chemical screen		10.1021/cb300047 4
38				behavior of seven glucocorticoids, eight androgens, and nine progestogens compared to six estrogens in a municipal sewage treatment plant (STP) in Beijing, China.	> Among all of the hormones considered, androgens were the dominant steroids detected in all samples (total concentrations up to 10 216 ± 912 ng/L for influents, 171 ± 10 ng/L for effluents, and 647 ± 52 ng/g for dehydrated sludge samples), followed by estrogens (102 ± 8 ng/L, 14 ± 2 ng/L, and 14 ± 1 ng/g), progestogens (57 ± 6 ng/L, 8 ± 2 ng/L, and 13 ± 3 ng/g), and glucocorticoids (42 ±2 ng/L, 0.7 ±0.1 ng/L, and 1.2 ± 0.3 ng/g). > With the exception of 19-nor-4-androstene-3,17-diol (NAD, 67%), removal rates for androgens were relatively high (98 99%), while those for glucocorticoids, estrogens, and all progestogens except 6R-methylhydroxyprogesterone (MHPT) were 85 - 99%, 78 - 99%, and 73 - 96%, respectively. > Glucocorticoids, androgens, and progestogens were mainly removed by degradation as with estrogens, while different behaviors were observed in the aerated grit chamber, anaerobic tank, anoxic tank, and aerobic tank units.	Behaviors of Glucocorticoids, Androgens and Progestogens in a Municipal Sewage Treatment Plant: Comparison to Estrogens	Fan et al. / Environ. Sci. Technol., 2011, 45 (7), pp 2725–2733
39	zebrafish larvae	dexamethasone, prednisolone and triamcinolone	50 pM to 50 nM	exposure from 3 h to 5 days post-fertilisation	>Microarray analysis identified 1255, 1531, and 2380 gene probes, which correspondingly mapped to 660, 882 and 1238 human/rodent homologs, as deregulated by dexamethasone, prednisolone and triamcinolone, respectively. >A total of 248 gene probes which mapped to 159 human/rodent homologs were commonly deregulated by the three glucocorticoids. These homologs were associated with over 20 molecular functions from cell cycle to cellular metabolisms,	Common deregulated gene expression profiles and morphological changes in developing zebrafish larvae exposed to environmental-relevant high to low concentrations of glucocorticoids	Q. Chen et al. / Chemosphere 172 (2017) 429e439

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					and were involved in the development and function of connective tissue, nervous, haematological, and digestive systems. >Glucocorticoid receptor signalling, NRF2-mediated oxidative stress response and RAR signalling were among the top perturbed canonical pathways. >Morphological analyses using four transgenic zebrafish lines revealed that the hepatic and endothelial-vascular systems were affected by all three glucocorticoids while nervous, pancreatic and myeloid cell systems were affected by one of them. >Quantitative real-time PCR detected significant change in the expression of seven genes at 50 pM of all three glucocorticoids, a concentration comparable to total glucocorticoids reported in environmental waters.		
40	rainbow trout	beclomethasone dipropionate (BDP) and monopropionate (BMP), beclomethasone	1, 10 and 100 ng/L	14 day flow-through exposure	>Beclomethasone (up to 0.65 µg/L) was not taken up in the fish while BDP (0.65 and 0.07 µg/L) resulted in accumulation of both beclomethasone, BMP and BDP in plasma, reaching levels up to those found in humans during therapy. >Accordingly, exposure to 0.65 µg/L of BDP significantly increased blood glucose as well as oxidized glutathione levels and catalase activity in the liver. >Exposure to beclomethasone or the low concentration of BDP had no effect on these endpoints. >Both exposure concentrations of BDP resulted in significantly higher transcript abundance of phosphoenolpyruvate carboxykinase involved in gluconeogenesis, and of genes involved in immune responses. >As only the rapidly metabolized prodrug was	Waterborne beclomethasone dipropionate affects the physiology of fish while its metabolite beclomethasone is not taken up	B.M. Carney Almroth et al. / Science of the Total Environment 511 (2015) 37–46

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					potent in fish, the environmental risks associated with the use of BDP are probably small.		
41	fathead minnow	mixtures of: one estrogen, EE2; one androgen, trenbolone; one glucocorticoid, beclomethasone dipropionate; and two progestogens, desogestrel and levonorgestrel	three sets of mixtures with low (0.16-300 ng/L), medium (0.5-1159.4 ng/L) and high (1-2317 ng/L) concentrations	21 days exposure, female egg production	> egg production EC50: Beclomethasone 596 ng/L, Desogestrel 2253 ng/L, Levonorgestrel 9.11 ng/L > The mixture inhibited egg production in an additive manner >A significant combined effect was observed when each steroidal pharmaceutical in the mixture was present at a concentration which on its own would produce no statistically significant effect (something from 'nothing'). >Further, when each component was present in the mixture at a concentration expected to inhibit egg production by between 18% (Beclomethasone diproprionate) and 40% (trenbolone), this mixture almost completely inhibited egg production	The consequences of exposure to mixtures of chemicals: Something from 'nothing' and 'a lot from a little' when fish are exposed to steroid hormones	T.J. Thrupp et al. / Science of the Total Environment 619–620 (2018) 1482–1492
42	Common carp	dexamethasone	1 mg L−1	The effects of dexamethasone on a host–pathogen (Common carp and A. salmonicida) relationship were analyzed.	>One hundred percent mortality was observed in bacteria-infected fish exposed to dexamethasone, whereas no infection-associated mortality was observed in infected fish in the absence of dexamethasone exposure. >In a separate experiment, dexamethasone exposure significantly suppressed hemolytic complement activity in bacteria-infected fish. >These results clearly indicate that exposure to a high concentration of dexamethasone suppressed the carp immune system and caused subsequent mortality	Use of common carp (Cyprinus carpio) and Aeromonas salmonicida for detection of immunomodulatory effects of chemicals on fish	K. Nakayama et al. / Marine Pollution Bulletin 124 (2017) 710–713
43	zebrafish larvae	prednisolone	0.1-10 µg/L	Physiological and behavioural responses Light-dark box test, Avoidance of visual stimulus	>Although exposure to prednisolone did not alter the morphology of the external eye, aggregation of melanin within the skin in response to increasing light levels was impeded and embryos exposed to prednisolone (10 µg/l) maintained a darkened phenotype.	Environmental concentrations of prednisolone alter visually mediated responses during early	McNeil et al. / Environmental Pollution 218 (2016) 981e987

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					>Exposure to prednisolone also increased the preference of embryos for a dark environment within a light dark box test in a concentration dependent manner. >However the ability of embryos to detect motion appeared unaffected by prednisolone. >significant effects were detected in several processes mediated by vision, changes occurred in a manner which suggest that vision was in itself unaffected by prednisolone. >Neurological and endocrinological changes during early ontogeny are considered as likely candidates for future investigation.	life stages of zebrafish (Danio rerio)	
44	Japanese medaka	Betamethasone	0.01, 0.1, and 1.0 µg betamethasone/L	two-generation fish full life cycle study	>estimated betamethasone concentrations to be <0.6 ng/L in 95% of all U.S. surface waters. The highest concentration at which ED outcomes are not anticipated was determined to be 0.1 µg/L. >The ratio of the predicted environmental concentration to the no effect concentration for ED is less than one, indicating no risk to aquatic life from environmentally relevant concentrations of betamethasone.	The endocrine disruption potential of betamethasone using Japanese medaka as a fish model	Vestel et al / Doi 10.1080/10807039.2017.1292841
45 (see Serial No. 71)	rainbow trout, Hepatic microsomes	clotrimazole and dexamethasone	1-or 50 µM	interactions between the phytochemicals and pharmaceuticals, single and in combination	>Singly, clotrimazole inhibited EROD activity 40% and 90% of control, while dexamethasone did not. >Naringenin and diosmin inhibited EROD activity alone up to 90% and 55% respectively, but activities were further inhibited in the presence of either pharmaceutical. >The preliminary study of combinations of clotrimazole with phytochemicals primarily showed synergistic effects. While EROD activity was not inhibited in the presence of quercetin or indole-3-carbinol, significant and synergistic inhibition was	In vitro effects of diosmin, naringenin, quercetin and indole-3-carbinol on fish hepatic CYP1A1 in the presence of clotrimazole and dexamethasone	S. Sakalli et al. / Chemosphere 192 (2018) 105e112

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
					detected when either of these was combined with clotrimazole or dexamethasone.		
46	Hoplias malabaricus	diclofenac and dexamethasone	diclofenac 0.2 mg/kg, 2.0 mg/kg, or 20.0 mg/kg or dexamethasone 0.03 mg/kg, 0.3 mg/kg, or 3.0 mg/kg	trophic exposure to 12 doses	>Diclofenac and dexamethasone altered the red blood cell count, as well as hematocrit and hemoglobin levels. >The total blood leukocyte count decreased in all groups. A significantly reduced carrageenan-induced leukocyte migration to the peritoneal cavity, particularly of polymorphonuclear cells, was observed at all tested doses, suggesting a possible immunosuppressive effect. >The basal nitric oxide synthesis of head kidney cell cultures was reduced at the highest dose of diclofenac and was increased at the highest dose of dexamethasone. The lipopolysaccharide-stimulated nitric oxide production was reduced in all treatments, thus corroborating the immunosuppressive effect.	Effects of trophic exposure to diclofenac and dexamethasone on hematological parameters and immune response in freshwater fish	Ribas, J. L. C., Zampronio, A. R. and Silva de Assis, H. C. (2016), Environ Toxicol Chem, 35: 975–982. doi:10.1002/etc.3240
47	zebrafish embryo	progesterone (P4), dexamethasone (DEX), 17a-ethinyl estradiol (EE2), testosterone (TTR), levonorgestrel (LNG), gestodene (GES), dydrogesterone (DDG), drospirenone (DRS), medrox-yprogesterone acetate (MPA), norethindrone acetate (NET),	0.1 nM, 10 nM, and 1 µM	exposure 7 dpf, locomotor behavior and the transcriptional levels of core clock genes	>progestins and corticosteroids, including progesterone and cortisol, significantly decreased the locomotor activities of eleuthero-embryos at concentrations as low as 16 ng/L, while estrogens such as 17b-estradiol led to an increase. >Consistently, progestins and corticosteroids displayed similar transcriptional effects on core clock genes, which were remarkably different from those of estrogens. >Of these genes, per1a and nr1d2a displayed the most pronounced alterations. >They were induced upon exposure to various progestins and corticosteroids and could be recovered using the progesterone receptor/glucocorticoid receptor antagonist mifepristone; this, however, was not the	Regulation of zebrafish (Danio rerio) locomotor behavior and circadian rhythm network by environmental steroid hormones	Y. Zhao et al. / Environmental Pollution 232 (2018) 422-429

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
		cortisol (CRL), prednisone (PRE), prednisolone (PREL), betamethasone (BET), fludrocortisone acetate (FLU), estrone (E1), 17b-estradiol (E2), estriol (E3), androstenedione (ADD) and androsterone (ADR).			case for estrogens and the estrogen receptor antagonist 4-hydroxy-tamoxifen		
48	gilthead seabream (Sparus aurata)	dexamethasone		cloned and sequenced a 1028 bp 50-flanking DNA region from the IL-6 gene Functional characterization of sbIL-6P was performed by cloning sbIL-6P into a luciferase expression vector and by transfecting it into L6 muscle cells, a mammalian cell line shown previously to express IL-6 in response to pro	>transcriptional regulation of the Interleukin-6 (IL-6) gene in fish is not well understood >seabream IL-6 promoter (sbIL-6P) evidenced the presence of a conserved TATA motif and conserved response elements for NF-kB, C/EBPb (NF-IL6), AP-1 and GRE, similar to other vertebrate IL-6 promoters >the activity of sbIL-6P was significantly induced by pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFa), IL-6 and IL-2, as well as by lipopolysaccharide (LPS), but significantly repressed by dexamethasone.	Transcriptional regulation of the gilthead seabream (Sparus aurata) interleukin-6 gene promoter	B. Castellana et al. / Fish & Shellfish Immunology 35 (2013) 71-78

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
				inflammatory stimuli			
49	zebrafish embryos	clobetasol propionate (CLO), cortisol and cortisone	0.1, 1, 10, and 100 µg/L	120 hpf, exposure of single compounds and binary mixtures	>CLO and cortisol, but not cortisone showed a concentration dependent decrease in muscle contraction, increase in heart rate, and accelerated hatching. >CLO also induced immobilization and edema at high concentrations. Transcription analysis covering up to 26 genes showed that mostly genes related to glucose metabolism, immune system and development were differentially expressed at 91 ng/L and higher. >CLO showed stronger effects on immune system genes than cortisol, which was characterized by upregulation of fkbp5, irg1l, gilz, and socs3, and development genes, matrix metalloproteinases mmp-9 and mmp-13, while cortisol led to stronger upregulation of the gluconeogenesis genes g6pca and pepck1. >CLO also induced genes regulating the circadian rhythm, nr1d1 and per1a. > In contrast, cortisone led to down-regulation of vitellogenin. >Binary mixtures of cortisol and CLO mostly showed a similar activity as CLO alone on physiological and transcriptional end points but additive effects in heart rate and pepck1 upregulation, which indicates that mixtures of glucocorticoids may be of concern for developing fish	Active Glucocorticoids Have a Range of Important Adverse Developmental and Physiological Effects on Developing Zebrafish Embryos	Willi et al. / DOI: 10.1021/acs.est.7b 06057 Environ. Sci. Technol. 2018, 52, 877–885
50	Ceriodaphnia dubia	dexamethasone (DEX) and prednisolone (PDS)	1.95 -125 µg/L for DEX; 7.8 -2000 µg/L for PDS	48-h acute immobilisation tests and Multi-generational test	>C. dubia exhibited varied sensitivities towards both the studied chemicals however were more sensitive to DEX with 48-h EC50 of 0.75 mg/L in comparison to PDS [19 mg/L]. >EC10 values for F0 in a multigenerational chronic	Multigenerational effects of two glucocorticoids (prednisolone and dexamethasone) on	N. Bal et al. / Environmental Pollution 225 (2017) 569e578

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
				to up to 4 generations	bioassay were 48 mg/L for DEX and 460 mg/L for PDS and in F3 were 2.2 mg/L for DEX and 31 mg/L for PDS. >There was a positive trend of increased toxicity followed by reduced life history traits such as fecundity, brood size and time to first brood and intrinsic rate of population increase and body growth (length and area) of C. dubia in the case of both studied chemicals.	life-history parameters of crustacean Ceriodaphnia dubia (Cladocera)	
51	Physa acuta (freshwater snail)	prednisolone (PDS)	2 - 64 µg/L	exposure to different life-cycle stages and multiple generations	>This continuous exposure over a period of multiple generations resulted in generational impairments at measured endpoints. > LOEC values ($p < 0.001$) for PDS exposure ranged from 32 to 4 µg/L in exposed F0-F2 generations. >Global DNA methylation (% 5-methyl cytosine) of adult progeny was found to be affected at higher test concentrations in comparison to the parent snails. > Partially formed to completely missed growth components of shell structure and shell thinning in abnormally underdeveloped PDS exposed snails of F1 and F2 generation, was also observed in this multigenerational exposure experiment	Assessing multigenerational effects of prednisolone to the freshwater snail, Physa acuta (Gastropoda: Physidae)	N. Bal et al. / Journal of Hazardous Materials 339 (2017) 281–291
52	zebrafish	Prednisolone	0.1, 1, and 10 µg/L	exposure for 120 hpf	>The frequency of spontaneous muscle contractions (24 hpf) was significantly reduced by prednisolone and 0.1 µg/L increased the distance embryos swam in response to a mechanosensory stimulus (48 hpf). > The percentage of embryos hatched significantly increased following prednisolone treatment (1 and 10 µg/L), while growth and mortality were unaffected. >The onset of heart contraction was differentially affected by prednisolone while heart rate and oxygen consumption both increased significantly	Physiological and Behavioral Effects of Exposure to Environmentally Relevant Concentrations of Prednisolone During Zebrafish (Danio rerio) Embryogenesis	McNeil et al./ DOI: 10.1021/acs.est.6b 00276 Environ. Sci. Technol. 2016, 50, 5294−5304

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					throughout embryogenesis. >Morphological changes to the lower jaw were detected at 96 hpf in response to 1 µg/L of prednisolone. >Several parameters of swim behavior were also significantly affected		
53	Chinook salmon	dexamethasone (DEX)		effects of chronic exposure to adrenocorticotropic hormone (ACTH) or the synthetic glucocorticoid dexamethasone (DEX) on the expression of genes involved in cortisol synthesis	DEX significantly reduced resting plasma cortisol levels and induced interrenal cell atrophy. Although no significant effect of treatment with DEX was found for any transcript, immunoreactivity for P450scc and P45011β appeared to be reduced	Effects of chronic manipulation of adrenocorticotropic hormone levels in Chinook salmon on expression of interrenal steroidogenic acute regulatory protein and steroidogenic enzymes	Henry J.McQuillan et al / General and Comparative Endocrinology Volume 174, Issue 2, 1 November 2011, Pages 156-165
54	Physa acuta (freshwater snail)	prednisolone	15.6 - 1000 µg/L	lethal and sublethal effects on the embryonic and posthatching stage	>Treatment with prednisolone at 125µg/L to 1000µg/L resulted in significant decline in growth, survival, and heart rate, as well as notable abnormalities in embryonic development. >Premature embryonic hatching was observed at lower concentrations of 31.25µg/L and 62.5µg/L, whereas delayed hatching was seen at concentrations from 125µg/L to 1000µg/L. >To assess impacts of prednisolone exposure on the hatched juveniles, the drug exposure was extended for another 28 d. Impairment of shell development was noted in juveniles exposed to concentrations from 62.5µg/L to 1000µg/L at the end of 42 d, which resulted in thin and fragile shells. >The thickness of shells in snails exposed to 1000µg/L was significantly lower in comparison to those in the 15.6-µg/L and control treatments. >	Prednisolone impairs embryonic and posthatching development and shell formation of the freshwater snail, Physa acuta	Bal, N., Kumar, A., Du, J. and Nugegoda, D. (2016), Environ Toxicol Chem, 35: 2339–2348. doi:10.1002/etc.3401

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					lower calcium concentration in shells of the exposed juvenile snails at treatments of 62.5µg/L to 1000µg/L consequently reduced their growth.		
55	GR-GeneBlazer Assay	effluent from municipal wastewater treatment (USA)		GR-GeneBlazer Assay	> The observed in vitro GR activity ranged from 39 to 155 ng dexamethasone equivalent/L (ng Dex-EQ/L) in the secondary effluents of four wastewater treatment plants. Monochromatic ultraviolet light of 80 mJ/cm2 disinfection dose was efficient for GR activity photolysis, whereas chlorination could not appreciably attenuate the observed GR activity. Ozonation was effective only at relatively high dose (ozone/TOC 1:1). Microfiltration membranes were not efficient for GR activity attenuation; however, reverse osmosis removed GR activity to levels below the limits of detection. > Twelve GR agonists were identified and quantified in effluents at summed concentrations of 9.6–21.2 ng/L. > The summed Dex-EQ of individual compounds based on their measured concentrations was in excellent agreement with the Dex-EQ obtained from bioassay, which demonstrated that the detected glucocorticoids can entirely explain the observed GR bioactivity. > Four synthetic glucocorticoids (triamcinolone acetonide, fluocinolone acetonide, clobetasol propionate, and fluticasone propionate) predominantly accounted for GR activity.	Balancing the Budget: Accounting for Glucocorticoid Bioactivity and Fate during Water Treatment	Ai Jia, Shimin Wu, Kevin D. Daniels, and Shane A. Snyder Environmental Science & Technology 2016 50 (6), 2870-2880 DOI: 10.1021/acs.est.5b04893
56	zebrafish	zygotic cortisol content		zygotic cortisol content was manipulated by microinjecting antibody to	>The resulting larval phenotypes revealed distinct treatment effects, including deformed mesoderm structures when maternal cortisol was unavailable and cardiac edema after excess cortisol. >Maternal cortisol unavailability heightened the	Maternal Cortisol Mediates Hypothalamus-Pituitary-Interrenal Axis	Nesan et al. / Scientific Reports volume 6, Article number: 22582 (2016)

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				sequester this steroid, thereby making it unavailable during embryogenesis. Compared with embryos containing excess cortisol by microinjection of exogenous steroid.	cortisol stress response in post-hatch larvae, whereas excess cortisol abolished the stressor-mediated cortisol elevation. >This contrasting hormonal response corresponded with altered expression of key HPI axis genes, including crf, 11B hydroxylase, pomca, and star, which were upregulated in response to reduced cortisol availability and downregulated when embryos had excess cortisol. >These findings for the first time underscore a critical role for maternally deposited cortisol in programming HPI axis development and function in zebrafish	Development in Zebrafish	doi:10.1038/srep22582
57	zebrafish	cortisol		Cortisol was microinjected into one-cell embryos	>Elevated embryo cortisol content increased heart deformities, including pericardial edema and malformed chambers, and lowered resting heartbeat post-hatch. >This phenotype coincided with suppression of key cardiac genes, including nkx2.5, cardiac myosin light chain 1, cardiac troponin type T2A, and calcium transporting ATPase, underpinning a mechanistic link to heart malformation. >The attenuation of the heartbeat response to a secondary stressor post-hatch also confirms a functional reduction in cardiac performance. >Altogether, high cortisol content during embryogenesis, mimicking increased deposition due to maternal stress, decreases cardiac performance and may reduce zebrafish offspring survival.	Embryo exposure to elevated cortisol level leads to cardiac performance dysfunction in zebrafish	D. Nesan, M.M. Vijayan / Molecular and Cellular Endocrinology 363 (2012) 85–91
58	zebrafish (8 months old)	fludrocortisone acetate (FLU)	0.006 and 42 µg/L	21-day exposure	>No remarkable reproductive impacts were observed, while physiological effects, including plasma glucose level and blood leukocyte numbers were significant altered even at 42 ng/L.	Corticosteroid Fludrocortisone Acetate Targets Multiple End Points in Zebrafish	Zhao et al. / Environ. Sci. Technol., 2016, 50

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					>Ovary parameters and transcriptional analysis of hypothalamic–pituitary–gonadal–liver axis revealed negligible effects. >Significant alterations of the circadian rhythm network were observed in the zebrafish brain. >Transcripts of several biomarker genes, including per1a and nr1d1, displayed strong transcriptional changes, which occurred at environmental relevant concentrations of 6 and 42 ng/L FLU. >Importantly, the development and behavior of F1 embryos were significant changed. >Heartbeat, hatching success and swimming behavior of F1 embryos were all increased even at 6 and 42 ng/L. All effects were further confirmed by exposure of eleuthero-embryos. >Significant transcriptional changes of biomarker genes involved in gluconeogenesis, immune response and circadian rhythm in eleuthero-embryos confirmed the observations in adult fish. Hatching success, heartbeat, and swimming activity were increased at 81 ng/L and higher, as with F1 embryos.	(Danio rerio) at Low Concentrations	(18), pp 10245–10254
59	zebrafish	dexamethasone		morpholinos, knock-down of both GR isoforms	GRb may act as a dominant-negative inhibitor of GRa when GRb is overexpressed and the GRa expression level is knocked down simultaneously. >without GRa knockdown, no evidence for this activity was found. In addition, the data indicate regulation of gene transcription through other mechanisms of action by GRb . >We found that dexamethasone treatment and knockdown of GRa togehter with over expression of GRb had opposite effects on glucose, amino acid, and fatty acid levels.	Transcriptional and Metabolic Effects of Glucocorticoid Receptor α and β Signaling in Zebrafish	Chatzopoulou et al. / Endocrinology, May 2015, 156(5):1757–1769

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60 (see Serial No. 25)							
61	zebrafish embryo	cortisol	0-5 µM	exposure for 21 days	>Treated larvae had elevated whole-body cortisol and glucocorticoid signaling, and upregulated genes associated with defense response and immune system processes. In adulthood the treated fish maintained elevated basal cortisol levels in the absence of exogenous cortisol, and constitutively mis-expressed genes involved in defense response and its regulation. > Adults derived from cortisol-treated embryos displayed defective tailfin regeneration, heightened basal expression of pro-inflammatory genes, and failure to appropriately regulate those genes following injury or immunological challenge. >These results support the hypothesis that chronically elevated glucocorticoid signaling early in life directs development of a pro-inflammatory adult phenotype, at the expense of immunoregulation and somatic regenerative capacity.	Cortisol-treated zebrafish embryos develop into pro-inflammatory adults with aberrant immune gene regulation	Hartig et al. / Biology Open 2016: bio.020065 doi: 10.1242/bio.020065 Published 21 July 2016
62	zebrafish, adult	prednisolone phosphate	25 µM	scales were removed and allowed to regenerate	> Prednisolone enhances osteoclast activity and matrix resorption and slows down the build up of the calcium/phosphorus molar ratio indicative of altered crystal maturation. > Prednisolone treatment further impedes regeneration through a shift in the time profiles of osteoblast and osteoclast genes that commensurates with an osteoporosis-like imbalance in bone formation. > A glucocorticoid-induced osteoporosis phenotype	Prednisolone induces osteoporosis-like phenotype in regenerating zebrafish scales	De Vrieze et al. / Osteoporos Int (2014) 25:567–578

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					as seen in mammals was induced in regenerating scalar bone of zebrafish treated with prednisolone.		
63	Brachionus calyciflorus, Thamnocephalus platyurus, Daphnia magna, Pseudokirchneriella subcapitata, Ceriodaphnia dubia	prednisolone, dexamethasone		Light exposure causes partial phototransformation; photoproducts tested in acute and chronic exposure	> The photochemical derivatives are more toxic than the parent compounds. > Generally low acute toxicity was found. Chronic exposure to this class of pharmaceuticals caused inhibition of growth population on the freshwater crustacean C. dubia while the alga P. subcapitata seems to be less affected by the presence of these drugs.	Toxicity of prednisolone, dexamethasone and their photochemical derivatives on aquatic organisms	DellaGreca et al. / Chemosphere 54 (2004) 629–637
64	Fathead Minnow	amioda-rone, carbamazepine, clozapine, dexamethasone, fenofi-brate, ibuprofen, norethindrone, or verapamil.		OECD 210 ELS	>Exposure of FHM to carbamazepine, fenofibrate, and ibuprofen resulted in no significant adverse effects at the concentrations tested. >FHM survival was not impacted by verapamil exposure; however, growth was significantly decreased at 600 µg/L. >Dexamethasone-exposed FHM showed a significant decrease in survival at a concentration of 577 µg/L; however, growth was not impacted at the concentration tested. >Norethindrone exposure resulted in a significant decrease in survival and dry weight at 14.8 and 0.74 µg/L, respectively. >Exposure to amiodarone and clozapine resulted in a significant decrease in survival and a significant increase in growth at concentrations of 1020 and 30.8 µg/L, respectively.	Early Life-Stage Toxicity of Eight Pharmaceuticals to the Fathead Minnow, Pimephales promelas	Overturf et al. / Arch Environ Contam Toxicol (2012) 62:455–464 DOI 10.1007/s00244-011-9723-6
65	Piaractus mesopotamicus	dexamethasone, levamisole and mixture of both	50 mg/kg of levamisole, 2.0 mg/kg of	accumulation of macrophages and formation of giant cells in chronic	> dexamethasone affects negatively the formation of giant cells in the chronic inflammation for foreign body. > Levamisole, despite being immunostimulatory in	Influence of dexamethasone and levamisole on macrophage	Petrillo, T. R. et al. / Biosci. J., Uberlândia, v. 33,

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
			dexamethasone	inflammation by foreign body and blood parameters in pacu	several species, showed limited action. >However, it was enough to counteract the effect of dexamethasone; the association of the drugs did not interfere significantly in erythrocyte and leukocyte number in most of the treatments and times studied. > In dexamethasone group there was a reduction in the number of erythrocytes and hemoglobin associated with increased mean corpuscular volume, suggesting slight macrocytic anemia. At 15 DPI, most groups showed the recovery of hematologic response. > Levamisole showed little effect by itself. However, in some parameters the association of both drugs causes similar response to control and naïve groups, showing the antagonistic effect of these drugs.	recruitment, giant cell formation and blood parameters in the tropical fish Piaractus mesopotamicus	n. 4, p. 1015-1027, July/Aug. 2017
66	brown trout (Salmo trutta)	cortisol	200 - 900 ng/mL	short-term (2 weeks) and long-term (4 months over winter)	>Cortisol caused an increase in glutathione over a 2-week period and appeared to reduce glutathione over winter. >Cortisol treatment did not affect oxidative stress levels or lowmolecular weight antioxidants. >Cortisol caused a significant decrease in growth rates but did not affect predation risk. >Over-winter survival in the stream was associated with low levels of oxidative stress and glutathione. >Thus, oxidative stressmay be amechanism by which elevated cortisol causes negative physiological effects.	Short-term and long-term effects of transient exogenous cortisol manipulation on oxidative stress in juvenile brown trout	Birnie-Gauvin et al. / Journal of Experimental Biology (2017) 220, 1693-1700 doi:10.1242/jeb.155465
67	rainbow trout (Oncorhynchus mykiss)	cortisol and cortisol-BSA		rainbow trout skeletal myotubes were stimulated	resulting in an early induction of reactive oxygen species (ROS). This production was not suppressed by transcription or translation inhibitors, suggesting non-genomic pathway involvement.	Cortisol Induces Reactive Oxygen Species through a Membrane	Espinoza et al. / Journal of Cellular Biochemistry

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
					> Moreover, myotube preincubation with RU486 and NAC completely suppressed cortisol- and cortisol-BSA-induced ROS production. Subcellular fractionation analysis revealed the presence of cell membrane glucocorticoid receptors. Finally, cortisol-BSA induced a significant increase in ERK1/2 and CREB phosphorylation, as well as in CREB-dependent transcriptional activation of the pgc1a gene expression. The obtained results strongly suggest that cortisol acts through a non-genomic glucocorticoid receptor-mediated pathway to induce ROS production and contribute to ERK/CREB/PGC1-a signaling pathway activation as stress compensation mechanisms.	Glucocorticoid Receptor in Rainbow Trout Myotubes	118:718–725 (2017)
68	zebrafish	prednisolone		activity and differentiation of osteoblasts, osteoclasts, and immune cells during ontogenetic growth, homeostasis, and regeneration of zebrafish bone	> Macrophage numbers are reduced in both larval and adult tissues, correlating with decreased generation of myelomonocytes and enhanced apoptosis of these cells. > In contrast, osteoblasts fail to proliferate, show decreased activity, and undergo incomplete differentiation. > Prednisolone treatment mitigates the number and recruitment of osteoclasts to sites of bone regeneration in adult fish. > In combination, these effects delay bone growth and impair bone regeneration	Immune Suppressive and Bone Inhibitory Effects of Prednisolone in Growing and Regenerating Zebrafish Tissues	Geurtzen, K et al. / ournal of Bone and Mineral Research, Vol. 32, No. 12, December 2017, pp 2476–2488
69	zebrafish, larvae	dexamethasone	12.5 µg/mL	exposure at 5 dpf for 24hrs; dexamethasone-induced zebrafish model of fatty liver and	>dexamethasone exposure caused significant increases of liver size and number of fish with hepatic steatosis at 6 dpf. >The increase of liver size caused by dexamethasone was significantly reversed by treatment with RU486, a GR antagonist, and by gene knock-down with a morpholino against the	Dexamethasone-induced hepatomegaly and steatosis in larval zebrafish	Yin et al. / J. Toxicol. Sci. Vol.42, No.4, 455-459, 2017

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				hepatomegaly was established	GR. >The dexamethasone-induced hepatic steatosis was also inhibited by treatment with RU486.		
70	rainbow trout liver slices	cortisol		liver immune function; expression of three cortisol-responsive genes	>Abundance of IL-1β mRNA was upregulated by LPS and suppressed by cortisol treatment in a time-dependent manner. While abundance of IgM mRNA was suppressed by cortisol treatment and stimulated by LPS, there were no effects of cortisol or LPS on abundance of Lyz mRNA. Abundance of hepcidin and LEAP-2A mRNA levels were suppressed by cortisol treatment and stimulated by LPS. > These results demonstrate that cortisol directly suppresses abundance of GR, IGF-1, IL-1β, IgM, hepcidin, LEAP-2A and SOCS-1 mRNA transcripts in the rainbow trout liver. >We report for the first time, a suppressive effect of cortisol (within 8 h of treatment) on hepcidin and LEAP-2A mRNAs in rainbow trout liver, which suggests that acute stress may negatively affect liver immune function in rainbow trout.	Effects of cortisol and lipopolysaccharide on expression of select growth-, stress- and immune-related genes in rainbow trout liver	Shepherd et al. / Fish Shellfish Immunol. 2018 Jan 8;74:410-418. doi: 10.1016/j.fsi.2018. 01.003.
71 (please see Serial No. 45)							
72	zebrafish	cortisol or dexamethasone	2.5 mM	uptake of radioactive cortisol by embryos during a 1 h submersion.	> the signal in chorionated embryos was 85% (exposure: 1-2 hpf) or 78% (exposure: 48-49 hpf) of the signal present in an equal volume medium. By comparing embryos measured without chorion, we found that 18-20% of the radioactivity present in chorionated embryos is actually bound to the	Kinetics of glucocorticoid exposure in developing zebrafish: A tracer study.	Steenbergen et al. / Chemosphere. 2017 Sep; 183:147-155. doi: 10.1016/j.

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					chorion or located in the perivitelline space. Consequently, embryonic tissue contains radioactivity levels of 60% of a similar volume of medium after 1 h incubation. > During early developmental stages (1-48 hpf) exposure of more than 24 h in cortisol was needed to achieve radio-activity levels similar to an equal volume of medium within the embryonic tissue and more than 48 h for dexamethasone. >During later developmental stages (48-96 hpf) initial uptake dynamics were similar, but showed a decrease of tissue radioactivity to 20% of an equal volume of medium after hatching, probably due to development and activation of the hypothalamic pituitary interrenal axis.		
73	Epithelioma papulosum cyprini	dexamethasone		infection with viral hemorrhagic septicemia virus (VHSV)	the titer of VHSV did not increase but instead decreased after dexamethasone treatment, suggesting that dexamethasone not only downregulates type I IFN but also affects certain factors that are necessary for VHSV replication. > An important effect of HSP90 on replication of RNA viruses and downregulation of HSP90 by glucocorticoids have been reported. >In this study, dexamethasone downregulated HSP90a expression in EPC cells that were stimulated with poly(I:C) or infected with VHSV. Furthermore, cells treated with an HSP90 inhibitor, geldanamycin, showed significantly decreased titers of VHSV, suggesting that HSP90 may be an important host component involved in VHSV replication, and HSP90 inhibition might be one of the causes for the observed reduction in viral titer caused by dexamethasone treatment.	Dexamethasone treatment decreases replication of viral hemorrhagic septicemia virus in Epithelioma papulosum cyprini cells.	Kim et al. / Arch Virol. 2017 May;162(5):1387-1392.

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
74	crucian carp (Carassius auratus)	dexamethasone		effects of dexamethasone on susceptibility of crucian carp to *Aeromonas hydrophila;* dexamethasone for 0 day (group D), 3 days (group C), 6 days (group B), and 9 days (group A)	>After that crucian carp were infected with A. hydrophila, crucian carp treated by dexamethasone had higher mortality (group A 95%, group B 76%, group C 31%) when compared to group D (4% mortality); the amount of pathogen inwas significantly increased (P < 0.05) in liver, kidney and spleen of fish in group A-C compared to group D. >These results implicated that higher susceptibility caused by dexamethasone may be induced by the decrease of lysozyme activity and the down-regulation of some immune genes.	Preliminary study on the relationship between dexamethasone and pathogen susceptibility on crucian carp (Carassius auratus).	Qi et al. / Fish Shellfish Immunol. 2016 Dec;59:18-24. doi: 10.1016/j.fsi.2016. 10.017.
75	zebrafish	dexamethasone (a synthetic GC), an antisense GC receptor (GR) morpholino (GR Mo)		120 h post fertilisation	>All treatments reduced cortisol levels in embryonic fish to similar levels. However, morpholino- and hypoxia-treated embryos showed delayed physical development (slower hatching and straightening of head–trunk angle, shorter body length), less locomotor activity, reduced tactile responses and anxiogenic activity. In contrast, dexamethasone-treated embryos showed advanced development and thigmotaxis but no change in locomotor activity or tactile responses. >Gene expression changes were consistent with increased (dexamethasone) and decreased (hypoxia, GR Mo) GC activity. >In adults, stressed cortisol values were increased with dexamethasone and decreased by GR Mo and hypoxia pre-treatments. Other responses were similarly differentially affected. In three separate tests of behaviour, dexamethasone-programmed fish appeared 'bolder' than matched controls, whereas Mo and hypoxia pre-treated fish were unaffected or more reserved. Similarly, the dexamethasone group but not the Mo or hypoxia	Early-life glucocorticoids programme behaviour and metabolism in adulthood in zebrafish.	Wilson et al. / Endocrinol. 2016 Jul;230(1):125-42. doi: 10.1530/JOE-15-0376

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					groups were heavier, longer and had a greater girth than controls.		
76	Japanese eel	Dexamethasone (DEX)		functional links from cortisol stimulation, to Akt activation, to the expression of the transcriptional factor, Ostf1 in Japanese eel gill cell cultures	>Using the synthetic glucocorticoid receptor agonist, dexamethasone (DEX), Ostf1 expression is found to be activated via glucocorticoid receptor (GR) and mediated by the Akt-GSK3β signaling pathway. >Pharmacological experiments using kinase inhibitors reveal that the expression of Ostf1 is negatively regulated by Akt activation. The inhibition of PI3K or Akt activities, by the specific kinase inhibitors (wortmannin, LY294002 or SH6), stimulates Ostf1 expression, while a reduction of GSK3β activity by LiCl reduces Ostf1 expression. Collectively, our report for the first time indicates that DEX can induce Ostf1 via GR, with the involvement of the Akt-GSK3β signaling pathway in primary eel gill cell cultures. The data also suggest that Ostf1 may play different roles in gill cell survival during seawater acclimation.	Dexamethasone (DEX) induces Osmotic stress transcription factor 1 (Ostf1) through the Akt-GSK3β pathway in freshwater Japanese eel gill cell cultures.	Chow et al / Biol Open. 2013 Mar 28;2(5):487-91. doi: 10.1242/bio.20134135.
77	Pacific salmon			Cryptobia salmositica is a pathogenic haemoflagellate of Pacific salmon	> The synthetic glucocorticoid, dexamethasone (Dex), also stimulated the replication of the parasite and mifepristone (RU486), a synthetic steroid that has glucocorticoid receptor (GR) antagonist properties, inhibited the stimulatory actions of both cortisol and Dex, when added to the medium at a concentration of 100 ng/ ml co-culture with cortisol or Dex. > Furthermore, the dose-dependent effects of glucocorticoids (cortisol and Dex) on the multiplication of the haemoflagellate were correlated with the initial size of the inocula.	Cortisol and dexamethasone increase the in vitro multiplication of the haemoflagellate, Cryptobia salmositica, possibly by interaction with a glucocorticoid receptor-like protein.	Li et al / Int J Parasitol. 2013 Apr;43(5):353-60. doi: 10.1016/j.ijpara.2012.11.009
78		chemical contaminants at		in vitro tests for hormone and	> in vitro bioassays revealed the occurrence of xenobiotic and steroid-like activities, including very	Identification of synthetic steroids in	Creusot et al / Environ Sci

Serial No.	Organism/ Test species/ Age	Substance tested	Concen-tration range	Experimental approach	Results / Conclusions	Title	Reference
		river sites located downstream from a pharmaceutical factory		dioxin receptor agonists and antagonists by using a panel of seven permanent cell lines, namely MELN, MDA-kb2, HG5LN-hPR, HG5LN-hMR, HG5LN-hPXR, and PLHC-1, allowing the specific detection of ligands of ER, AR/GR, PR, MR, PXR, and AhR	high glucocorticoid, antimineralocorticoid, progestogenic and pregnane X receptor (PXR)-like activities (µg standard-EQ/g of sorbent range), and weak estrogenic activity (ng E2-EQ/g of sorbent range). > Chemical analyses detected up to 60 out of 118 targeted steroid and pharmaceutical compounds in the extracts. In vitro profiling of occurring individual chemicals revealed the ability of several ones to act as agonist and/or antagonist of different steroids receptors. Mass balance calculation identified dexamethasone, spironolactone, and 6-alpha-methylprednisolone as major contributors to corticosteroid activities and levonorgestrel as the main contributor to progestogenic activities.	river water downstream from pharmaceutical manufacture discharges based on a bioanalytical approach and passive sampling.	Technol. 2014 Apr 1;48(7):3649-57. doi: 10.1021/es405313r
79	rainbow trout	dexamethasone (DEX)	3, 30, 300 and 3000 ng L -1	Exposure in a semi-static system over a period of 42 d; Using hepatic microsomes, we determined cytochrome 450 content, activities of ethoxyresorufin O-deethylase (EROD), p-nitrophenol hydroxylase (PNPH), 7-benzyloxy-4-trifluoromethylcoumarin O-debenzylase	> results showed that fish do not change the catalytic activity of CYP450-mediated reactions after high DEX concentration exposure. These results disagree with mammalian studies, where DEX is a well-known inducer of CYP450. > We showed a significant effect of DEX exposure on CYP450-mediated reactions (EROD, BCFOD, BQOD and PNPH) when expressed as amount of product formed per min per nmol total CYP450 at 3, 30 and 300 ng L 1 after 21 d exposure. Moreover, BFCOD and BQ activities showed matching trends in all groups. > Western blot analysis showed induction of CYP3A-like protein in the presence of the lowest environmentally relevant concentration of DEX	Does dexamethasone affect hepatic CYP450 system of fish? Semi-static in-vivo experiment on juvenile rainbow trout	Burkina, V. et al. / Chemosphere 139 (2015) 155–162

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				(BFCOD) and benzyloxyquinoline O-debenzylase (BQOD), as well as protein expression			

D.4 References of the literature research for glucocorticoids

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