Umwelt Bundesamt

As of: August 2023

Scientific Opinion on the safety evaluation of degradation products from stabilizers and derived MTC_{tap} values: current status of consent after consultations in Subgroup on Organic Materials (SG-OM) of 4MSI

Umweltbundesamt (UBA), Germany

ABSTRACT

This scientific opinion of UBA deals with the risk assessment of degradation products from stabilizers in plastics intended to come into contact with drinking water.

The migration of degradation products from stabilizers which are listed in Commission Regulation (EU) No. 10/2011 are mentioned in several citations (e.g.[1] [2])*). In order to assess the toxicological characteristics of ten significant substances of this group, UBA asked the association of stabilizer manufacturers (ELiSANA) to provide the necessary toxicological studies.

ELiSANA provided for most of the ten substances toxicological studies so that UBA in cooperation with the BfR (German Institute for Risk Assessment) could derive migration restrictions for them. This has been discussed in 4MSI-SG-OM and is documented in its current consent status here.

UBA recommends the inclusion of specific migration restrictions for these substances as additional requirement for plastics in the 4MSI Common Approach on Organic Materials in Contact with Drinking Water Part C - Procedures and Methods for Testing and Accepting Products Made of Organic Materials.

[1] D. Brocca et al. (Water Research 36, (2002), 3675–3680)

[2] D Löschner et al. (Anal. Methods 3, (2011), 2547–2556)

*): Degradation products are sometimes denoted as “Arvin Substances”, making reference to the corresponding author of early publications in this field. Some of the toxicological studies also use “Arvin” as substance identifier.

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SUMMARY

The literature describes the migration of degradation products from stabilizers from plastics, like plastic pipes, into drinking water. A group of ten well-known degradation products are considered here for toxicological evaluation, denoted by individual Roman numerals.

Table 1 Degradation products of stabilizers (sometimes called ”Arvin Substances“)

Substance	Name	CAS
I	4-Ethyl phenol	123-07-9
II	p-tert-Butylphenol	98-54-4
III	2,6-Di-tert-butyl-p-benzoquinone	719-22-2
IV	2,4-Di-tert-butyl phenol	96-76-4
V	3,5-Di-tert-butyl-4-hydroxy styrene	19263-36-6
VI	3,5-Di-tert-butyl-4-hydroxy benzaldehyde	1620-98-0
VII	3,5-Di-tert-butyl-4-hydroxy acetophenone	14035-33-7
VIII	7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	82304-66-3
IX	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)methyl propionate	6386-38-5
X	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propionic acid	20170-32-5

UBA conducted several migration tests with plastic pipes commercially available. Most of the ten substances were detected by GC/MS-screening in migration water samples. Only substance I (4-Ethylphenol), V (3,5-Di-tert-butylphenol) and VII (3,5-Di-tert-butyl-4-hydroxy acetophenone) could not be detected by this method.

Nevertheless, a toxicological assessment of these substances is regarded as necessary.

Although the stabilizers are assessed and listed in the context of Commission Regulation (EU) No. 10/2011, no restrictions for degradation products are set (except for substance II, p-tert-Butylphenol, listed with Ref. No 14020). Therefore, UBA asked the European association of stabilizer manufacturers (ELiSANA) to provide the necessary toxicological studies in order to assess these substances.

ELiSANA provided necessary genotoxicity tests for most of the ten substances, except for substance V. Additionally they provided oral studies for substances IV, IX and X.

UBA in cooperation with BfR derived migration restrictions (MTC_{tap}) which were subject to consultations within SG-OM. Substance identification and derived MTC_{tap} are given in Table 2.

Table 2 Derived migration restrictions for substances I through X

Subst. No.	Name	CAS	MTC_{tap} [µg/l]
I	4-Ethyl phenol	123-07-9	0.1
II	p-tert-Butylphenol	98-54-4	2.5
III	2,6-Di-tert-butyl-p-benzoquinone	719-22-2	2.5
IV	2,4-Di-tert-butyl phenol	96-76-4	250
V	3,5-Di-tert-butyl-4-hydroxy styrene	19263-36-6	0.1
VI	3,5-Di-tert-butyl-4-hydroxy benzaldehyde	1620-98-0	2.5
VII	3,5-Di-tert-butyl-4-hydroxy acetophenone	14035-33-7	2.5
VIII	7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	82304-66-3	100
IX	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)methyl propionate	6386-38-5	50 (as sum for both)
X	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propionic acid	20170-32-5	

As a result of observed occurrence of these substances and evaluation of relevant toxicological studies, UBA recommends the inclusion of MTC_{tap} restrictions tabulated above into the 4MSI Common Approach on Organic Materials in Contact with Drinking Water Part C - Procedures and Methods for Testing and Accepting Products Made of Organic Materials.

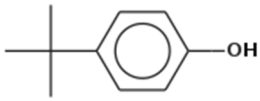
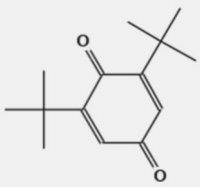
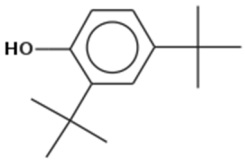
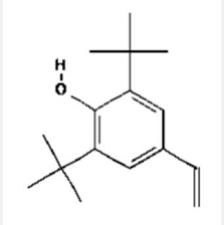
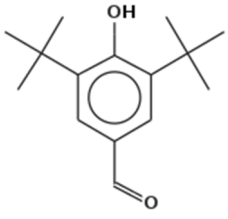
ASSESSMENT

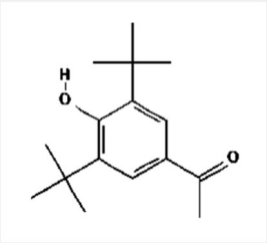
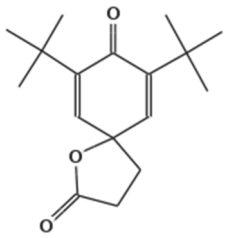
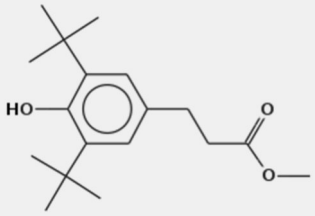
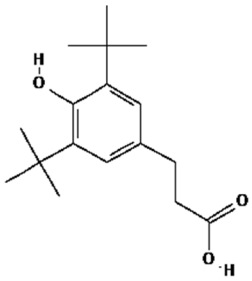
1 Identity

The following substances are degradation products from stabilizers used to produce plastic products intended to come into contact with drinking water. The stabilizers themselves are listed in Commission Regulation (EU) No. 10/2011, but there are no restrictions for corresponding degradation products.

This assessment only focuses on the selected suite of substances tabulated below.

Table 3 Structural formulas of substances I through X

Substance	Name	CAS	Structure
I	4-Ethyl phenol	123-07-9	
II	p-tert-Butylphenol	98-54-4	
III	2,6-Di-tert-butyl-p-benzoquinone	719-22-2	
IV	2,4-Di-tert-butyl phenol	96-76-4	
V	3,5-Di-tert-butyl-4-hydroxy styrene	19263-36-6	
VI	3,5-Di-tert-butyl-4-hydroxy benzaldehyde	1620-98-0	

Substance	Name	CAS	Structure
VII	3,5-Di-tert-butyl-4-hydroxy acetophenone	14035-33-7	
VIII	7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	82304-66-3	
IX	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)methyl propionate	6386-38-5	
X	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propionic acid	20170-32-5	

2 Physical and chemical properties

Molecular weight and aqueous solubility of substances, as available in safety data sheet, are presented in Table 4.

Table 4 Key physical properties of substances I through X

Subst. No.	Name	CAS	Molecular weight [g/mol]	Solubility in water
I	4-Ethylphenol	123-07-9	122.17	4900 mg/l (25 °C, measured)
II	p-tert-Butylphenol	98-54-4	150.22	610 mg/l (25 °C, OECD 105)
III	2,6-Di-tert-butyl-p-benzoquinone	719-22-2	220.31	5.8 mg/l (25°C, predicted EPI Suite)
IV	2,4-Di-tert-butylphenol	96-76-4	206.32	12 mg/l (20 °C, measured)
V	3,5-Di-tert-butyl-4-hydroxystyrene	19263-36-6	232.36	2.8 mg/l (25°C, predicted EPI Suite)
VI	3,5-Di-tert-butyl-4-hydroxybenzaldehyde	1620-98-0	234.33	28.4 mg/l (25°C, predicted EPI Suite)
VII	3,5-Di-tert-butyl-4-hydroxyacetophenone	14035-33-7	248.37	25.6 mg/l (25°C, predicted EPI Suite)
VIII	7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	82304-66-3	276.37	15.5 mg/l (25°C, predicted EPI Suite)
IX	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)methyl propionate	6386-38-5	292.42	2.2 mg/l (25 °C, OECD 105)
X	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propionic acid	20170-32-5	278.39	12.9 mg/l (25°C, predicted EPI Suite)

3 Intended Use

Not applicable

4 Authorization of the substances

No authorization

5 Substance migration

Migration tests were performed according to EN 12873-1 with cold and hot water. Test specimens were various commercially available plastic pipes for domestic installation. GC/MS-screening according to EN 15768 was used as analytical method and 34 different samples were tested.

The conversion factor used to calculate c_{tap} was 20 d/dm.

The results of the migration tests are given in Table 5.

Table 5 Results from migration testing for substances I through X

Subst. No.	Name	CAS	UBA (GC/MS-screening)	
			C_{tap} [µg/l], 23°C	C_{tap} [µg/l], 60°C
I	4-Ethylphenol	123-07-9	n.d.	n.d.
II	p-tert-Butylphenol	98-54-4	0.1	0.1-0.2
III	2,6-Di-tert-butyl-p-benzoquinone	719-22-2	0.02 – 0.6	0.16 – 4.8
IV	2,4-Di-tert-butylphenol	96-76-4	0.3 – 6.2	0.46 – 28.8
V	3,5-Di-tert-butyl-4-hydroxystyrene	19263-36-6	n.d.	n.d.
VI	3,5-Di-tert-butyl-4-hydroxybenzaldehyde	1620-98-0	0.01 – 1.7	0.1 – 3.8
VII	3,5-Di-tert-butyl-4-hydroxyacetophenone	14035-33-7	n.d.	n.d.
VIII	7,9-Di-tert-butyl-1-oxaspiro [4.5]deca-6,9-diene-2,8-dione	82304-66-3	0.04 – 2.9	1.7 – 49.8
IX	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)methyl propionate	6386-38-5	0.1 – 4.1	0.1 – 29.2
X	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propionic acid	20170-32-5	n.d.	0.17 – 17.7

Substances I, V and VII were not detectable. Substances II, III and VI in most cases remained below 2.5 µg/l, with few samples exceeding 2.5 µg/l but often displaying relatively poor sample quality in these cases. Substances IV, VIII, IX and X displayed concentrations above 2.5 µg/l.

Additional tests with LC/MS and single substance calibration revealed that using the GC/MS-screening method leads to an underestimation of substances VIII and X. Further tests with LC/MS on the different substances migrating from plastic pipes are in progress.

6 Residual Content

Not applicable

7 Microbiological properties

Not applicable

8 Toxicological Data

8.1 Substance I (CAS 123-07-9)

Although substance I could not be detected in the migration water of plastic pipes using GC/MS screening, ELiSANA provided genotoxicity tests.

Bacterial reverse mutation assay

The test was carried out in 2008 according to GLP and OECD 471 with and without metabolic activation. Source: summarized data (in English) from Japanese studies, URL: http://dra4.nihs.go.jp/mhlw_data/home/file/file123-07-9.html *[Feb. 2019, verified July 2021]*

Table 6 Bacterial reverse mutation assay for substance I

Test substance	
physical nature and purity	4-Ethylphenol 98.3 %
stability of test substance	stable at room temperature
Solvent/Vehicle	
justification for choice of solvent	DMSO was chosen because of solubility of test substance and relative non-toxicity to bacteria
solubility/stability	No data given
Strains	
straines used	TA 1535, TA 1537, TA 98, TA 100, Escherichia coli WP2 uvrA
Test conditions	
amount of test substance per plate	0, 62.5, 125, 250, 500, 1000, 2000 µg/plate performed in duplicate
metabolic activation system	phenobarbital and 5,6-benzoflavon induced rat liver cell S9-mix
Results	
	• no precipitation • toxicity occurred at 1000 µg/plate • positive and negative controls were carried out • carried out controls are in the range of historical controls • no relevant increase of the revertants with and without metabolic activation

Conclusion: There is no evidence for mutagenicity from this test.

***in vivo* Micronucleus test**

The test was carried out in 2016 according to OECD 474 and GLP.
Source: WIL Research Project 510774 - Test item 206749/A, "Micronucleus test in bone marrow cells of the mouse with Arvin #1", final report.

Table 7 *In vivo* Micronucleus test for substance I

Test substance	
physical nature and purity	4-Ethylphenol 99.0%
stability of test substance	stable at room temperature
Solvent/Vehicle	
justification for choice of solvent	corn oil was chosen because of good solubility and compatibility with test system
solubility/stability	No data given
Test animals	
animals used	NMRI mice (SPF)
number, age and sex	For each dose 5 male and 5 female, 6-7 weeks old
Test conditions	
dose level	125, 250 and 500 mg/kg male 187, 375 and 750 mg/kg female
route, duration of administration and sampling	two treatments with 24 h interval, gastric intubation, euthanized after 48 hours after the first treatment
target organs investigated	femora
Results	
	• animals of the highest dose were lethargic and had rough coat and hunched posture, also at the second treatment of female mice of dose group 375 mg/kg • no decrease in PCE/NCE ratio • no increase of micronuclei in PCE, except positive control • carried out controls are in the range of historical controls

Conclusion: There is no evidence for chromosomal damage from this test

The study report data of the *in vivo* micronucleus test cannot prove that the substance concentration at the target organ (bone marrow) was sufficiently high over the test time. Therefore, clastogenicity and aneugenicity cannot be excluded for Arvin I. Hence, a MTC_{tap} of **0.1 µg/l** results in view of possible genotoxic effects.

8.2 Substance II (CAS 98-54-4)

p-tert-Butylphenol (substance II) is assessed and listed in Commission Regulation (EU) No. 10/2011 with an SML of 50 mg/kg. This results in a MTC_{tap} of **2.5 µg/l**.

8.3 Substance III (CAS 719-22-2)

c_{tap} derived from migration tests of substance III in most cases remained below 2.5 µg/l. Therefore, it is regarded as sufficient to show that substance III is not genotoxic.

Bacterial reverse mutation assay

The test was carried out in 2016 according to GLP and OECD 471 with and without metabolic activation.
Source: WIL Research Project 510765 - Test item 206750/A, "Evaluation of the mutagenic activity of Arvin #3 in the Salmonella typhimurium reverse mutation assay and the Escherichia coli reverse mutation assay", final report.

Table 8 Bacterial reverse mutation assay for substance III

Test substance	
physical nature and purity	2,6-Di-tert-butyl-1,4-benzoquinone 100 %
stability of test substance	stable at room temperature
Solvent/Vehicle	
justification for choice of solvent	Ethanol was chosen because of solubility of test substance and relative non-toxicity to bacteria
solubility/stability	No data given
Strains	
straines used	TA 1535, TA 1537, TA 98, TA 100, Escherichia coli WP2 uvrA
Test conditions	
amount of test substance per plate	0, 52, 164, 512, 1600 and 5000 µg/plate performed in duplicate
metabolic activation system	Aroclor induced rat liver cell S9-mix
Results	
	• Precipitation at the start of the incubation period at concentrations of 512 µg/plate and upwards and at 5000 µg/plate at the end of the incubation period • no toxicity • positive and negative controls were carried out • carried out controls are in the range of historical controls • no relevant increase of the revertants with and without metabolic activation

Conclusion: There is no evidence for mutagenicity from this test.

***in vitro* Micronucleus test**

The test was carried out in 2016 according to OECD 487 and GLP.
Source: WIL Research Project 510770 - Test item 206750/A, "An *in vitro* micronucleus assay with Arvin #3 in cultured peripheral human lymphocytes", final report.

Table 9 *In vitro* Micronucleus test for substance III

Test substance	
physical nature and purity	2,6-Di-tert-butyl-1,4-benzoquinone 100 %
stability of test substance	stable at room temperature
Solvent/Vehicle	
justification for choice of solvent	Ethanol was chosen because of good solubility and compatibility with test system
solubility/stability	No data given
Strains	
straines used	peripheral human lymphocytes
Test conditions	
amount of test substance per plate	Exp. 1: 0, 10, 50, 70, 90, 110, 130 and 150 µg/ml (±S9) Exp. 2: 0, 10, 50, 70, 80, 90 and 100 µg/ml (±S9) (3 hours exposure time, 27 hours harvest time) Exp. 3: 5, 10, 20, 30, 40, 50 and 75 µg/ml (-S9) (24 hours exposure time,24 hours harvest time)
metabolic activation system	Phenobarbital and β-Naphthoflavone induced rat liver cell S9-mix
Results	
	• solvent control showed mono- and binucleated cells with micronuclei within range of the historical negative control • positive and negative control were in the range of historical data • no statistically significant and biologically relevant increase in the number of mono- and binucleated cells with micronuclei in the absence and presence of S9-mix

Conclusion: There is no evidence for clastogenic or aneugenic effects from this test

Substance III does not appear as genotoxic. Hence, a MTC_{tap} of **2.5 µg/l** would result.

8.4 Substance IV (CAS 96-76-4)

The expected concentration of substance IV at the tap is considerably higher than 2.5 µg/l. Therefore, it is necessary to have oral dose toxicity data in addition to genotoxicity data.

8.4.1 Genotoxicity data

Table 10 compilation of data for mutagenicity evaluation of Arvin IV

Test	Specification	Result
In vitro		
Ames Test	With and without metabolic activation, concentration tested 8 – 5000 µg/plate just IUCLID (2000) with short information to Ames-Test (Hüls AG Marl; Report-No. AM-91/4)**, study was not available**	negative, but not verifiable
Ames Test	With and without metabolic activation, concentration tested 8 – 5000 µg/plate According to ECHA registration dossier Ames-Test according to OECD 471 (2015), **study was not available**	negative, but not verifiable
Ames Test	Japanese „Ministry of Health, Labour and Welfare“, report only in Japanese, just an short English summary and English result tables available	negative, but not verifiable
Read across and *in silico* assessment to exclude mutagenicity due to missing valid data		
Read Across	2,4-Di-tert-amylphenol (EFSA Journal 2015;13(9):4242)	negative
In-silico-Assessment	Arvin IV with Derek Nexus (version 6.0.1) and Sarah Nexus (version 3.0.0)	negative

Conclusion: There is no evidence for mutagenicity from the read across and the *in silico* assessment, which correlates with the reported but not verifiable test results.

***in vivo* Micronucleus test**

The test was carried out in 2008 according to OECD 474 and GLP. Source: Charles River, Report-No: 29665.

Table 10 *In vivo* Micronucleus test for substance IV

Test substance	
physical nature and purity	2,4-di-tert-butyl phenol, 99%
stability of test substance	stable at room temperature, stored in the dark
Solvent/Vehicle	
justification for choice of solvent	corn oil was chosen because of good solubility and compatibility with test system
solubility/stability	Almost unsoluble in water
Test animals	
animals used	CD rat
number, age and sex	Control group consisted of 5 male and 5 female rats, the low and middle dose groups each consisted of 5 female rats the highest dose group consisted of 10 male and 10 female rats. The positive control group consisted of 5 male rats. 6-7 weeks old
Test conditions	
dose level	200, 400 and 800 mg/kg female 1000 mg/kg male (high dose)
route, duration of administration and sampling	two treatments with 24 h interval, oral route, euthanized after 48 hours after the first treatment
target organs investigated	femora
Results	
	• No animal deaths occurred following dosing. Clinical signs of hunched, subdued behaviour, wet around anus, wet staining pergenital, wet faeces, piloerection, salivation red discharge (nose), laboured breathing and staggering were observed • no decrease in PCE/NCE ratio • no increase of micronuclei in PCE, except positive control • carried out controls are in the range of historical controls

Conclusion: There is no evidence for chromosomal damage from this test

For the assessment of genotoxicity, only the original data of an *in vivo* micronucleus test was presented by the applicant (Charles River, Report-No: 29665). References to data of the REACH dossier or to an Ames test given in an IUCLID data sheet cannot be applied for assessment due to missing original study data. Accordingly, valid data for mutagenicity to assess the endpoint genotoxicity of substance IV was not available. Therefore, and to support the results of the *in vivo* micronucleus assay a read across to the structurally related 2,4-di-tert-amylphenol was carried out to assess the potential of genotoxicity for substance IV. 2,4-di-tert-amylphenol has been assessed by EFSA (EFSA Journal 2015;13(9):4242) with no mutagenic and no clastogenic activity. The read across is additionally supported by negative

results of an *in silico* assessment of substance IV using Derek Nexus (version 6.0.1) and Sarah Nexus (version 3.0.0).

Overall, 2,4-Di-tert-butylphenol is considered as not genotoxic.

8.4.2 Oral toxicity data

One generation oral study

The test was carried out in 1980[3]

Table 11 One generation study for substance IV

Test substance	
physical nature and purity	2,4-Di-tert-butyl phenol, >97%
stability of test substance	stable at room temperature, stored in the dark
Solvent/Vehicle	
justification for choice of solvent	corn oil was chosen because of good solubility and compatibility with test system
solubility/stability	Almost unsoluble in water
Test animals	
animals used	CD spraque dawley rats
age	9 weeks
acclimatisation	2.5 weeks
Body weight	317-362 g male 205-249 g female
Number and sex	F0: 15 animals per sex and dose group F1: 20 animals per sex and dose group, 5 of each sex and dose group had a recovery phase of 4 weeks after the 13 weeks administration
Test conditions	
dose level	0, 50, 150, 300 mg/kg·d
route, duration of administration and sampling	Oral route, diet mix continuously available, 28 weeks before mating, throughout mating gestation and lactation in females and F0 males during mating and until conception was established in females. F1 generation for 13 weeks.
Results	
Mortality F0	• One animal of highest dose has to be killed, pallor of all internal organs, discolouration of liver, kidney and lymph nodes, prominent splenic white pulp

[3] Hazleton Laboratories Europe Ltd., 1980: 2,4-Di-tert-butyl-phenol: F0/F1 generation oral (dietary toxicity study in the rat)

	• One animal died of pregnancy, one foetus in a difficult position for birth, gestation period 2.5 days longer • No other mortalities
Mortality F1	• One animal died directly after blood sampling at day 0 and was replaced immediately
Clinical observation	• No treatment related
Body weight F0	• Reduced body weight gain in high and • middle dose group
Body weight F1	• body weight of progeny from all treated groups significantly lower than controls • all treated groups lower body weight gain than control
Reproductive function	• unimpaired, but reduction of mean number of progeny born at 300 mg/kg. • liter loss in control group and 50 mg/kg and 300 mg/kg dose groups higher than usual in the strain
Haematology	• further evidence for groth retardation at high dose group animals, but blood chemistry and urine constituents did not reveal any signs of organ dysfunction
Organ weight	• dose dependent increase of liver weight at 150 and 300 mg/kg dose group, reversible • increased weight of brain, testes, thyroid and pituitary
Histopatholgy	• no changes in microscopic structure of organs and tissue which were unequivocally attributable to the adminsitration

Conclusion: No observed effect level given as 150 mg/kg body weight.

In the F0/F1 generation feeding study Sprague-Dawley-rats got 50, 150 and 300 mg/kg·d 2,4-Di-tert-butylphenol. The treatment started with the F_0-Generation 28 days prior to mating and continued until lactation of the female animals. The F1 generation (20 animals per dose and sex) were exposed in utero and until week 13 via feeding. A recovery phase of 4 weeks was performed with 5 animals per sex per dose of them. Primary toxic effects could be seen in the growth of the 300 mg/kg·d dose group of the F1-Generation. An increased liver weight was observed in the 150 mg/kg d and 300 mg/kg·d dose groups. The increased liver weights are seen as adaptive due to the missing dysfunction and the missing changes in the microscopic structure. Microsomal protein synthesis is assumed as reason for that. In the high dose group a decreased number of progeny and a reduced growth rate of them was observed. Therefore, a NOAEL of 150 mg/kg·d was determined.

A sub-acute study[4] was performed for comparison of the effects in newborn and juvenile rats. 15 newborn rats per dose group were fed from day 4 to 21 with 5, 40 and 150 mg/kg·d. The body weight was reduced up to 15 % - 25 % for male and 9 %- 20 % for female animals. Histopathological effects as well as organ weight changes and changes in blood chemistry were observed for liver and kidney. A NOAEL of 5 mg/kg·d was determined, 4 – 5fold lower than for the juvenile rats.
This sub-acute study is not sufficient to assess chronic exposure, but is taken into account as auxiliary result to help in validation of endpoints.

The F0/F1-generation study from 1980 is employed as the key study for MTC_{tap} derivation. A NOAEL of 150 mg/kg·d as determined in this study should be lowered due to (i) doubts on the interpretation of described hepato- and nephrological effects as adaptive and (ii) due to missing data on phospholipd- and γ-glutamyltransferase titers which would help in clarification. It is to be noted that generally liver and kidney appear as target organs in oral studies with substance IV and that somewhat elevated γ-glutamyltransferase values had been observed in the 28-day study [4]. To resume, a NOAEL of 50 mg/kg d is considered for substance IV.

8.4.3 Assessment of bioaccumulation

Due to missing toxicokinetic data for substance IV ELiSANA notes that in EFSA Journal 2015, 13(9):4242, estimates are given on metabolisation and subsequent excretion of twofold substituted alkylphenols as glucuronides and sulfates. These findings indicate that bioaccumulation of this substance group should be relatively low. An additional uncertainty factor of 3 is considered in this publication to allow for transfer of metabolisation findings to structural analogues.

8.4.4 MTC_{tap} calculation

The MTC_{tap} calculation is based on the read across to 2,4-di-tert-amylphenol, regarding the conclusions on deduction of lacking genotoxicity and bioaccumulation potential, and the NOAEL of 50 mg/kg·d from the F0/F1 generation oral subchronic oral toxicity study (Hazleton Ltd. 1980). The typical inter- and intraspecies factors totaling to a factor of 100, a factor of 2 for subchronic to chronic extrapolation, and an additional uncertainty factor of 3 for the read across and the uncertainty regarding the metabolism of 2,4-di-tert-butylphenol results in a tolerable concentration of 83.3 µg/kg·d (50 mg/ kg·d /(100x2x3)).
Assuming 60 kg body weight,a consumption of 2 liters water per day and a 10% allocation, an acceptable water concentration of 250 µg/l results. Thus, the maximum possible MTC_{tap} with regard to the available toxicological data of **250 µg/l** can be applied for 2,4-Di-tert-butylphenol.

[4] Hirata-Koizumi, M. et al. 2005: Elevated susceptibility of newborn as compared with young rats to 2-tert-butylphenol and 2,4-di-tert-butylphenol toxicity. Congenital Anomalies, 45, 146 - 153.

8.5 Substance V (CAS 19263-36-6)

Substance V is not included in the NIST database used to analyze GC/MS results and thus cannot be identified directly in migration tests performed at UBA. Moreover, in most articles dealing with migration of stabilizers from drinking water contact materials it was also not detected, except that it was mentioned in the original publication of Brocca and coworkers. This does not exclude possible appearance of substance V in plastic leachates since it is used as grafted polymer chain stabilizer (in-situ copolymerization).

No toxicological data was provided by industry.

Therefore, it is decided to apply a MTC_{tap} of **0.1 µg/l** for substance V.

8.6 Substance VI (CAS 1620-98-0)

The migration of substance VI in most cases leads to c_{tap} below 2.5 µg/l. Therefore, it is seen sufficient to show that substance VI is not genotoxic.

Bacterial reverse mutation assay

The test was carried out in 2016 according to GLP and OECD 471 with and without metabolic activation.
Source: WIL Research Project 510766 - Test item 206751/A, "Evaluation of the mutagenic activity of Arvin #6 in the Salmonella typhimurium reverse mutation assay and the Escherichia coli reverse mutation assay", final report.

Table 12 Bacterial reverse mutation assay for substance VI

Test substance	
physical nature and purity	3,5-Di-tert-butyl-4-hydroxybenzaldehyde 98.9 %
stability of test substance	stable at room temperature
Solvent/Vehicle	
justification for choice of solvent	DMSO was chosen because of solubility of test substance and relative non-toxicity to bacteria
solubility/stability	Almost unsoluble in water
Strains	
straines used	TA 1535, TA 1537, TA 98, TA 100, Escherichia coli WP2 uvrA
Test conditions	
amount of test substance per plate	1.Exp.: 0, 1.7, 5.4, 17, 52, 164, 512, 1600, 2500 µg/plate (TA 100, WP2uvrA) 0, 52, 164, 512, 1600, 2500 µg/plate (TA1535, TA1537, TA98) 2. Exp. 0, 154, 275, 492, 878, 1568 µg/plate Tests were performed in triplicate

metabolic activation system	Aroclor induced rat liver cell S9-mix
Results	
	• Precipitation at the start of the incubation period at concentrations of 512 µg/plate and upwards and at 1600 and 2500 µg/plate at the end of the incubation period • no toxicity • positive and negative controls were carried out • carried out controls are in the range of historical controls • no relevant increase of the revertants with and without metabolic activation

Conclusion: There is no evidence for mutagenicity from this test.

***in vitro* Micronucleus test**

The test was carried out in 2016 according to OECD 487 and GLP.
Source: WIL Research Project 510771 - Test item 206751/A, "An *in vitro* micronucleus assay with Arvin #6 in cultured peripheral human lymphocytes", final report.

Table 13 *In vitro* Micronucleus test for substance VI

Test substance	
physical nature and purity	3,5-Di-tert-butyl-4-hydroxybenzaldehyde 98.9 %
stability of test substance	stable at room temperature
Solvent/Vehicle	
justification for choice of solvent	DMSO was chosen because of good solubility and compatibility with test system
solubility/stability	Almost unsoluble in water
Strains	
strains used	peripheral human lymphocytes
Test conditions	
amount of test substance per plate	1. Exp.: 0, 5.4, 17, 52 µg/ml (±S9) (3 hours exposure time, 27 hours harvest time) 2. Exp.: 0, 5.4, 17, 52 µg/ml (±S9) (24 hours exposure time, 24 hours harvest time) Tests performed in duplicate
metabolic activation system	Phenobarbital and β-Naphthoflavone induced rat liver cell S9-mix
Results	
	• precipitation at 52 µg/ml • solvent control showed mono- and binucleated cells with micronuclei within range of the historical negative control

	• positive and negative control were in the range of historical data • no statistically significant and biologically relevant increase in the number of mono- and binucleated cells with micronuclei in the absence and presence of S9-mix

Conclusion: There is no evidence for clastogenic or aneugenic effects from this test

Substance VI is regarded as not genotoxic. Hence, a MTC_{tap} of **2.5 µg/l** would result.

8.7 Substance VII (CAS 14035-33-7)

The migration of substance VII in most cases leads to c_{tap} below 2.5 µg/l. Therefore, it is sufficient to show that substance VII is not genotoxic.

Bacterial reverse mutation assay

The test was carried out in 2016 according to GLP and OECD 471 with and without metabolic activation.
Source: WIL Research Project 510767 - Test item 206752/A, "Evaluation of the mutagenic activity of Arvin #7 in the Salmonella typhimurium reverse mutation assay and the Escherichia coli reverse mutation assay", final report."

Table 14 Bacterial reverse mutation assay for substance VII

Test substance	
physical nature and purity	3',5'-Di-tert-butyl-4'-hydroxyacetophenone 99.4 %
stability of test substance	stable at room temperature
Solvent/Vehicle	
justification for choice of solvent	DMSO was chosen because of solubility of test substance and relative non-toxicity to bacteria
solubility/stability	Almost unsoluble in water
Strains	
straines used	TA 1535, TA 1537, TA 98, TA 100, Escherichia coli WP2 uvrA
Test conditions	
amount of test substance per plate	1.Exp.: 0, 1.7, 5.4, 17, 52, 164, 512, 1600, 2500 µg/plate (TA 100, WP2uvrA) 0, 17, 52, 164, 512, 1600 µg/plate (TA1535, TA1537, TA98) 2. Exp. 0, 154, 275, 492, 878, 1568 µg/plate Tests were performed in triplicate
metabolic activation system	Aroclor induced rat liver cell S9-mix
Results	

	• Precipitation at the start of the incubation period at concentrations of 164 µg/plate and upwards and at 1600 µg/plate at the end of the incubation period • no toxicity • positive and negative controls were carried out • fluctuation of revertant colonies below historical control data in Exp. 1 • in Exp. 2 carried out controls are in the range of historical controls • slight to extreme reductions in the number of revertant colonies in TA100 at 512 µg/plate and above in the absence of S9-mix and at 5000 µg/plate in the presence of S9-mix. In the other tester strains, no biologically relevant decrease in the number of revertants were observed. • no relevant increase of the revertants with and without metabolic activation

Conclusion: There is no evidence for mutagenicity from this test.

***in vitro* Micronucleus test**

The test was carried out in 2016 according to OECD 487 and GLP.
Source: WIL Research Project 510772 - Test item 206752/A, "An *in vitro* micronucleus assay with Arvin #7 in cultured peripheral human lymphocytes", final report.

Table 15 *In vitro* Micronucleus test for substance VII

Test substance	
physical nature and purity	3',5'-Di-tert-butyl-4'-hydroxyacetophenone 99.4 %
stability of test substance	stable at room temperature
Solvent/Vehicle	
justification for choice of solvent	DMSO was chosen because of good solubility and compatibility with test system
solubility/stability	Almost unsoluble in water
Strains	
strains used	peripheral human lymphocytes
Test conditions	
amount of test substance per plate	1. Exp.: 0, 17, 52, 164 µg/ml (±S9) (3 hours exposure time, 27 hours harvest time) 2. Exp.: 0, 17, 52, 164 µg/ml (±S9) (24 hours exposure time, 24 hours harvest time) Tests performed in duplicate
metabolic activation system	Phenobarbital and β-Naphthoflavone induced rat liver cell S9-mix

Results	
	• precipitation at 164 µg/ml • solvent control showed mono- and binucleated cells with micronuclei within range of the historical negative control • positive and negative control were in the range of historical data • no statistically significant and biologically relevant increase in the number of mono- and binucleated cells with micronuclei in the absence and presence of S9-mix

Conclusion: There is no evidence for clastogenic or aneugenic effects from this test

Substance VII is regarded as not genotoxic. Hence, a MTC_{tap} of **2.5 µg/l** would result.

8.8 Substance VIII (CAS 82304-66-3)

Although it is reported that the open-chain form of substance VIII might undergo ring closure due to the hot injector of a GC/MS, a LC/MS test showed, that the "oxa-spiro"- structure is migrating from materials. This was confirmed by additional NMR spectra of the standard used for calibration. The LC/MS tests additionally showed, that the migration of substance VIII is underestimated by the GC/MS-screening method. The migration values might be twice as high as measured with the semi-quantitative method.
Therefore, oral toxicity data is necessary in addition to the genotoxicity data.

ELiSANA assumed that substance VIII is transformed to its open-chain structure in the gastro-intestinal tract. They proposed to do a read across to similar open-chain substances.

Until now it could not be proven that a ring opening takes place. Nevertheless, the proposed "read across" to substance X (3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propionic acid) is not considered as applicable, as the open-ring-form of substance VIII is an α,β- unsaturated ketone with hydroxyl-, alkyl- and alkyl carbonic acid side chains, whereas substance X is a phenol with alkyl and alkyl carbonic acid side chains.
In conclusion, and in view of c_{tap} values for substance VIII markedly exceeding 2.5 µg/l, an additional 90-day oral study is requested by UBA.

8.8.1 Genotoxicity data

Bacterial reverse mutation assay

The test was carried out in 2016 according to GLP and OECD 471 with and without metabolic activation.
Source: WIL Research Project 510768 - Test item 207010/A, "Evaluation of the mutagenic activity of Arvin #8 in the Salmonella typhimurium reverse mutation assay and the Escherichia coli reverse mutation assay", final report."

Table 16 Bacterial reverse mutation assay for substance VIII

Test substance	
physical nature and purity	7,9-Di-tert-butyl-1-oxa-spiro[4.5]deca-6,9-dien-2,8-dion >98 %
stability of test substance	stable at room temperature
Solvent/Vehicle	
justification for choice of solvent	DMSO was chosen because of solubility of test substance and relative non-toxicity to bacteria
solubility/stability	Almost unsoluble in water
Strains	
straines used	TA 1535, TA 1537, TA 98, TA 100, Escherichia coli WP2 uvrA
Test conditions	
amount of test substance per plate	1.Exp.: 0, 1.7, 5.4, 17, 52, 164, 512, 1600, 2500 µg/plate (TA 100, WP2uvrA) 0, 17, 52, 164, 512, 1600 µg/plate (TA1535, TA1537, TA98) 2. Exp. 0, 154, 275, 492, 878, 1568 µg/plate Tests were performed in triplicate
metabolic activation system	Aroclor induced rat liver cell S9-mix
Results	
	• Precipitation at the start and end of the incubation period at concentrations of 492 µg/plate and upwards • no toxicity • positive and negative controls were carried out • fluctuation of revertant colonies of TA 100 below historical control data in both Exp. • other controls are in the range of historical controls • no relevant increase of the revertants with and without metabolic activation

Conclusion: There is no evidence for mutagenicity from this test.

***in vitro* Micronucleus test**

The test was carried out in 2016 according to OECD 487 and GLP.
Source: WIL Research Project 510773 - Test item 207010/A, "An *in vitro* micronucleus assay with Arvin #8 in cultured peripheral human lymphocytes", final report.

Table 17 *In vitro* Micronucleus test for substance VIII

Test substance	
physical nature and purity	7,9-Di-tert-butyl-1-oxa-spiro[4.5]deca-6,9-dien-2,8-dione ›98 %
stability of test substance	stable at room temperature
Solvent/Vehicle	
justification for choice of solvent	DMSO was chosen because of good solubility and compatibility with test system
solubility/stability	Almost unsoluble in water
Strains	
strains used	peripheral human lymphocytes
Test conditions	
amount of test substance per plate	1. Exp.: 0, 15, 90, 110 µg/ml (±S9) (3 hours exposure time, 27 hours harvest time) 2. Exp.: 0, 15, 70, 110 µg/ml (±S9) (24 hours exposure time, 24 hours harvest time) Tests performed in duplicate
metabolic activation system	Phenobarbital and β-Naphthoflavone induced rat liver cell S9-mix
Results	
	• precipitation at 164 µg/ml • solvent control showed mono- and binucleated cells with micronuclei within range of the historical negative control • positive and negative control were in the range of historical data • no statistically significant and biologically relevant increase in the number of mono- and binucleated cells with micronuclei in the absence and presence of S9-mix

Conclusion: There is no evidence for clastogenic or aneugenic effects from this test

As the drinking water relevant substance VIII is identical with 7,9-Di-tert-butyl-1-oxa-spiro[4.5]deca-6,9-dien-2,8-dione, it can be regarded as not genotoxic.

8.8.2 Oral toxicity data

In 2023, a 90-day oral feeding study in Wistar rats according to OECD TG 408 with Arvin VIII (oxaspiro variant) was finalized and submitted (Table 19). The test substance Arvin VIII was given by gavage in 3 dose groups of 20, 100 and 500 mg/kg bw/ day. The control group and the high dose group consisted of 20 male and 20 female animals, because these groups contained animals to be examined after a recovery period. The low and mid dose group consisted of 10 male and 10 female rats, respectively.
Substance identity is well documented by chemical analyses certificates in the study report. Historical control data is not included for all substance related effect endpoints.

90-day oral study

The test was reported 2023 according to OECD 408 and GLP.
Source:
Report: 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, Repeated Dose 90-Day Oral Toxicity Study in Wistar Rats including a Recovery Period of at least 28 Days, BASF, Report; Project No.: 50C0217/20C015,

Table 19 90-day oral study for substance VIII

Test substance	
physical nature and purity	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, 99 %
stability of test substance	stable at room temperature
Solvent/Vehicle	
justification for choice of solvent	Corn Oil Ph.Eur. Grad, CAS No.: 8001-30-7
solubility/stability	stability of the test substance in corn oil over a period of 7 days at room temperature was demonstrated
Test animals	
animals used	Wistar rats (Crl:WI(Han))
age	6 weeks
acclimatisation	no data given
Body weight	172 +-15 g male 127 +-10 g female
Number and sex	20 animals per sex and dose group for control and high dose 10 animal per sex and dose group for low and mid dose
Test conditions	
dose level	0, 20, 100, 500 mg/kg*d
route, duration of administration and sampling	Intragastrically by gavage, 3 months
Results	
Mortality and clinical signs	• Decreased values in motor activity measurement in males and females at the end of the administration period in dose groups 100 and 500 mg/kg bw/day • Reduced prothrombin time (Hepatoquick's test, HQT) in males and females in dose groups 100 and 500 mg/kg bw/day • Increased T4 values in females in dose groups 100 and 500 mg/kg bw/day

	• Increased total protein and globulin values in males and females in dose group 500 mg/kg bw/day • Increased total cholesterol, LDL- and HDL-cholesterol values in males in dose group 100 and 500 mg/kg bw/day • Slight increase of total cholesterol in female in dose groups 20, 100 and 500 mg/kg bw/day (figure 6) • Decreased triglyceride values in males in dose group 500 mg/kg bw/day • Decreased glucose levels in females in dose group 500 mg/kg bw/day • Increased TSH values in males in dose group 100 and 500 mg/kg bw/day (figure 5) • Slight increased TSH values in males in dose group 20 mg/kg bw/day (figure 5)
Body weight	• Body weight gain of all animals similar to control group • Food consumption of all treated animals similar to control group
Organ weight	• liver weights increased significantly in dose groups 100 and 500 mg/kg bw/day • slight increase of liver weights in dose group 20 mg/kg bw/day (figure 2) • Increased absolute and relative weight of the thyroid glands in males in dose groups 100 and 500 mg/kg bw/day • slight increase of thyroid glands weights in dose group 20 mg/kg bw/day (figure 1)
Histopatholgy	• Increased incidence of hypertrophy of the hepatocytes at 100 and 500 mg/kg bw/day (figure 4) • one incidence of hypertrophy of the hepatocytes at 20 mg/kg bw/day • hypertrophy/hyperplasia of thyroid follicular cells and alteration of colloid (figure 4)

The study authors derived a NOAEL of 20 mg/kg bw * day, based on significant effects on absolute and relative liver weight, histopathological finding in liver and thyroid, absolute and relative thyroid weight, decreased motoric activity, T4 and TSH concentrations, as well as changed cholesterol levels. The most relevant findings of the study are summarized in figures 1 through 6.

First effects can already be seen at the low dose level with 20 mg/kg bw * day not only for the thyroid weight and histological finding, but also for the liver effects (Figure 1 -6). Thus, a dose dependency already starts at 20 mg/kg bw/day.
Due to these small but first effects already at the low dose and missing historical control data for the liver effects, the NOAEL is rather assessed as equivocal effects at the low dose level and might rather be a LOEL and an additional assessment factor of 3 is suggested accordingly.
This additional assessment factor of 3 is also supported by the histopathological effects in the thyroid gland, which may not be directly relevant to the human thyroid, but show an effect on the liver metabolism in rats already at the low dose level.

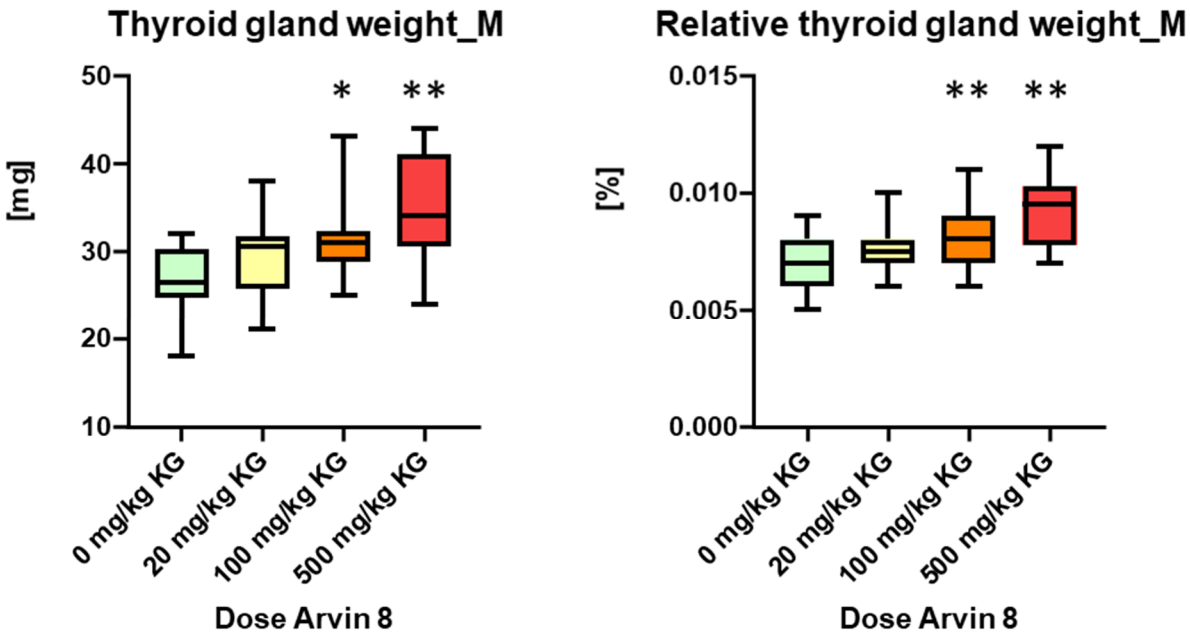


Figure 1 Dose dependency of adverse effect of Arvin VIII on thyroid gland in male rats

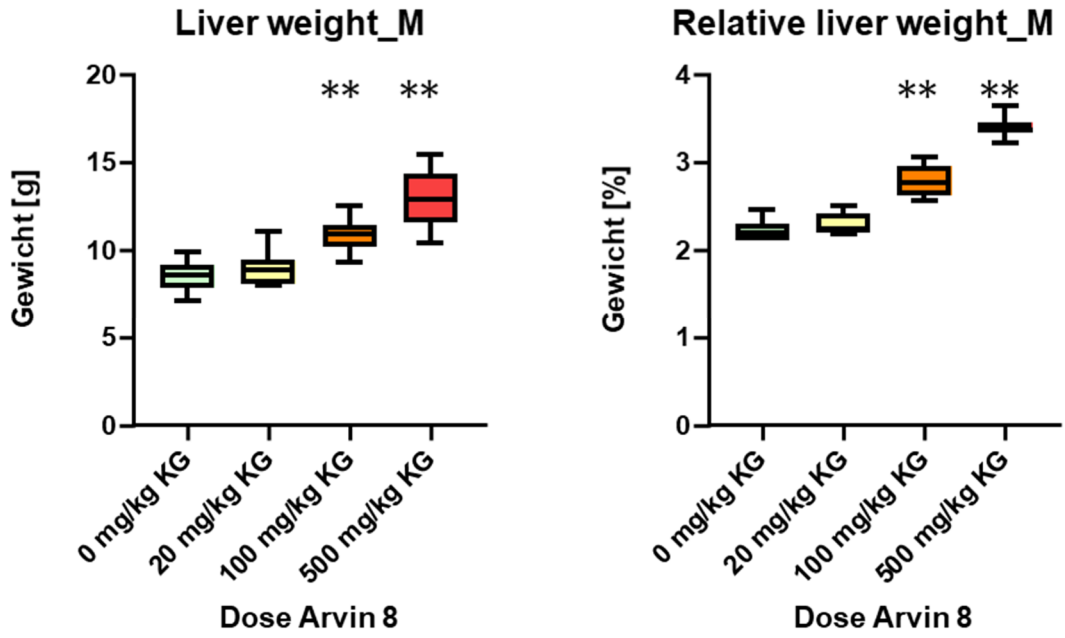


Figure 2 Dose dependency of adverse effect of Arvin VIII on liver weight in male rats

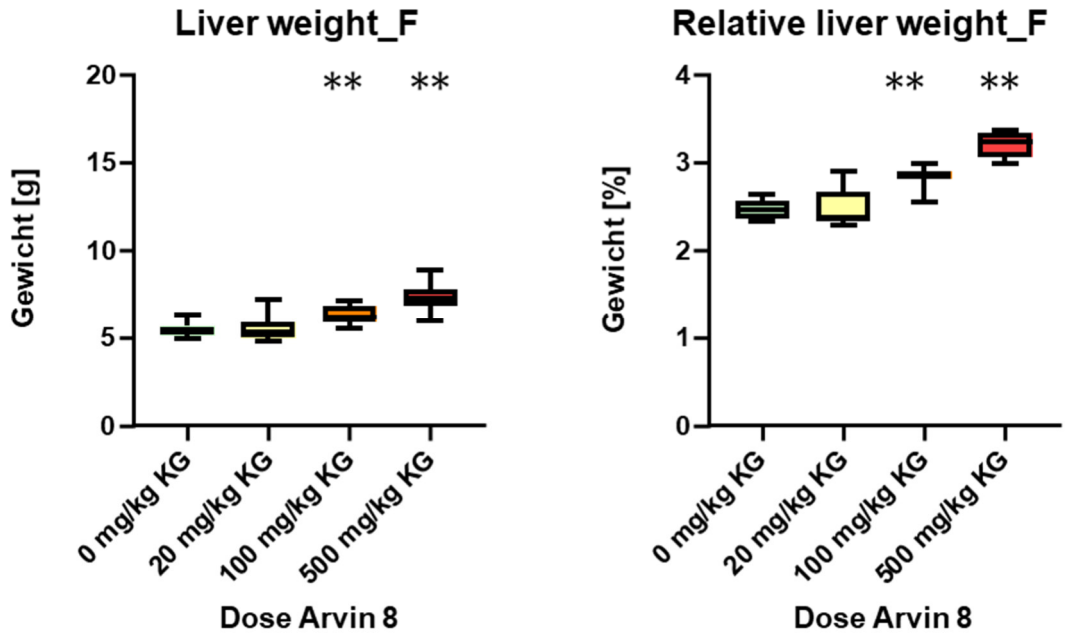


Figure 3 Dose dependency of adverse effect of Arvin VIII on liver weight in female rats

The boxes shown in the graphs of figure 1-3 indicate the 25th, 50th and 75th percentiles. The lower antenna marks the 10th, the upper the 90th percentile. Significance: * ≤ 0,05; ** ≤ 0,01

Removal Reason(s): ALL	Male				Female			
Summary: Incidence	Group 0 0 mg/kg	Group 1 20 mg/kg	Group 2 100 mg/kg	Group 3 500 mg/kg	Group 0 0 mg/kg	Group 1 20 mg/kg	Group 2 100 mg/kg	Group 3 500 mg/kg
Number of Animals:	10	10	10	10	10	10	10	10
LIVER								
Examined	10	10	10	10	10	10	10	10
Hypertrophy, hepatocellular, centrilobular	.	1	7	8	.	.	5	10
.... minimal	.	1	0	2	.	.	4	3
.... slight	.	0	6	2	.	.	1	6
.... moderate	.	0	1	4	.	.	0	1
THYROID GLANDS								
Examined	10	10	10	10	10	10	10	10
Hypertrophy/hyperplasia, follicular cell	0	1	8	10	1	0	3	3
.... minimal	0	0	1	2	1	0	3	3
.... slight	0	1	5	1	0	0	0	0
.... moderate	0	0	2	6	0	0	0	0
.... marked; severe	0	0	0	1	0	0	0	0
Alteration, colloid	2	8	9	10	0	1	4	5
.... minimal	2	7	3	0	0	1	4	5
.... slight	0	1	4	5	0	0	0	0
.... moderate	0	0	2	4	0	0	0	0
.... marked; severe	0	0	0	1	0	0	0	0

Figure 4 Dose dependency of adverse effect of Arvin VIII on histopathology of liver and thyroid gland in rats

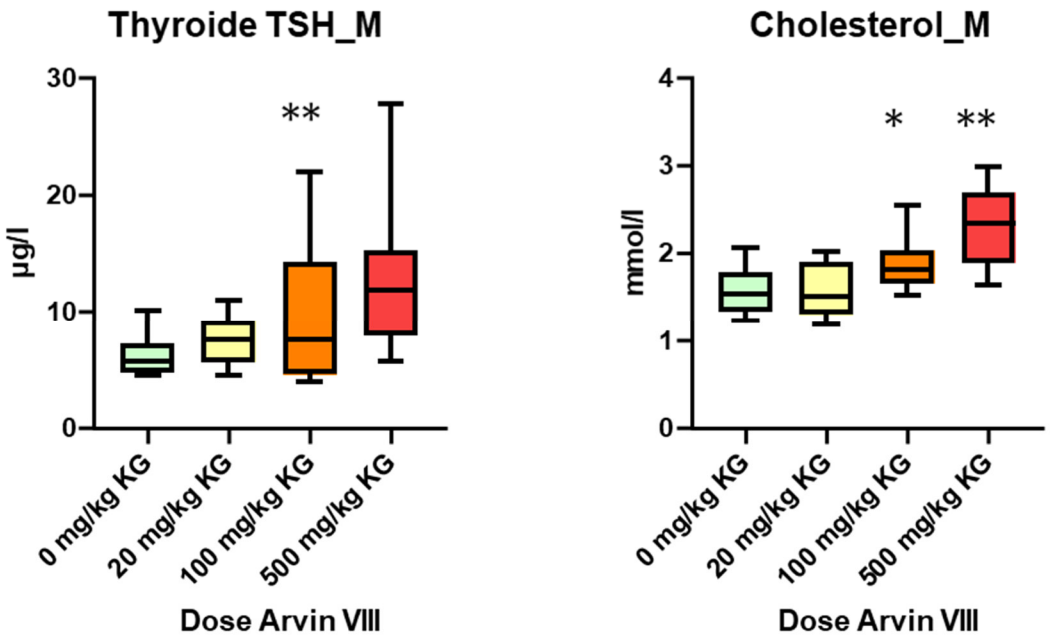


Figure 5 Dose dependency of adverse effect of Arvin VIII on TSH and cholesterol level in male rats

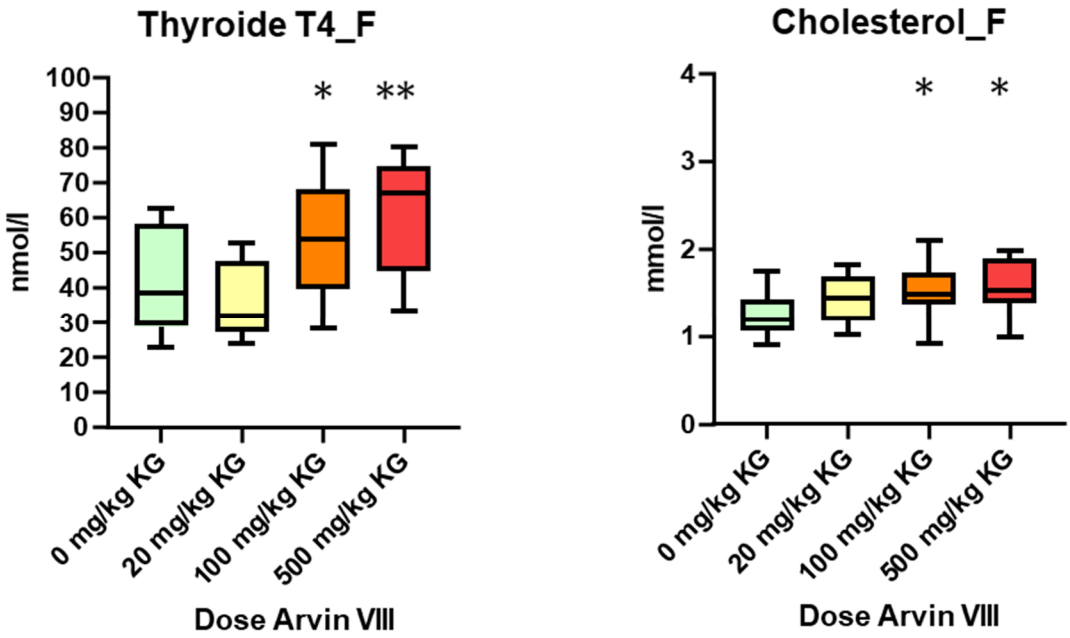


Figure 6 Dose dependency of adverse effect of Arvin VIII on T4 and cholesterol level in female rats

TDI derivation (extrapolation factors):

- inter species toxicodynamics and toxicokinetics :		10
- intra species toxicodynamics and toxicokinetics:		10
- subchronic to chronic:		2
- equivocal effects at low dose, missing historic control liver, liver effect, limited data on bioaccumulation potential:		3
- overall	=	600

TDI = 20 mg/kg bw/day / 600

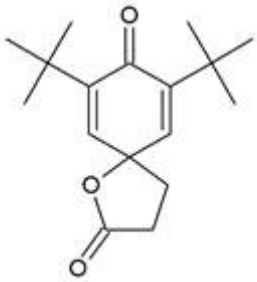
TDI = 0.033 mg/kg bw/day

8.8.3 Accumulation in Man

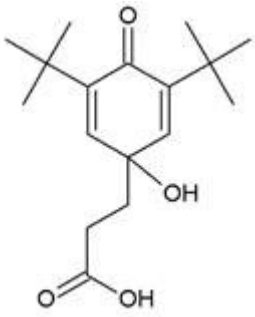
The log Kow value for substance VIII is 3.39 according to US EPA prediction (https://comptox.epa.gov/dashboard/chemical/properties/DTXSID60337884), and thus above 3, where toxicokinetic data is necessary for the assessment of its bioaccumulation potential in man. A separate toxicokinetic study with repeated substance administration, which would allow the best assessment, is not available.
Thus, the results from the recovery period of the 90-day oral study shall be assessed with regard to the bioaccumulation potential in man. Data from the recovery phase of the study at the high dose group show a significant reduction of adverse effects in rats and in most cases the adverse effects could not be detected anymore: “After a four-week recovery period, the female animals were fully recovered. In male animals dosed with 500 mg/kg bw/d prior to the recovery period, the increased thyroid gland organ weight has not yet fully normalized.”
This indicates that the substance VIII is readily removed by the rats after the administration had been stopped. Accordingly, the accumulation potential of substance VIII can be assessed as very low to not relevant. Nevertheless, due to not fully adequate study data on toxicokinetics an additional extrapolation factor may be warranted. As an extrapolation factor for data uncertainties has already been introduced for substance VIII, this factor of 3 shall also include the uncertainties regarding bioaccumulation in man.
Applying an extrapolation factor of 3 for remaining data uncertainties has already been applied and accepted for substances IV and IX/X, and thus, can be regarded as consistent throughout migrations substance assessment.

8.8.4 Research requirement on structure and transformation of Arvin VIII

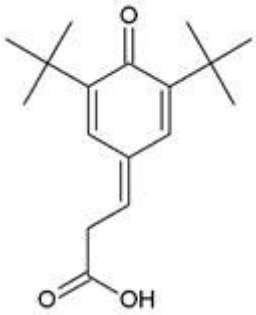
It has been shown by dedicated investigations (extraction of migration water at different pH, applying both GC/MS and LC/MS) that the hitherto anticipated lactonic structure of substance VIII is transformed to its open acidic form (substance VIII-acid) under certain conditions. This holds for moderately basic conditions and to a limited degree for GC/MS-analysis as well. The modes and prerequisites of transformation still have to be explored, but it appears that reliable estimation of Arvin VIII concentrations is more challenging than expected. In addition, the vinylic structure (propenoic acid derivative) originally assigned by Arvin and coworkers to substance VIII might also be present, since it is the dehydrated derivative (elimination of H_2O) of substance VIII-acid. For structural relationships cf. picture below.



Substance VIII



Substance VIII – acid



Substance VIII – en –acid

8.8.5 MTC_{tap} derivation

Arvin VIII has been assessed as not genotoxic based on negative effects in one OECD TG 471 and one OECD TG 487 study. Because migration of Arvin VIII is well above 2.5 µg/l, additionally a 90-day oral feeding study according to OECD TG 408 and an assessment as not accumulating in man is required to derive a MTC_{tap}.
Bioaccumulation of Arvin VIII in man is unlikely, due to the significant recovery after administration was stopped in the 90-day oral study.
The conducted 90-day oral feeding study lead to a TDI of 0.033 mg/kg bw/day.
The TDI derivation includes a total extrapolation factor of 600, consisting of factors of 10 for interspecies and 10 for intraspecies variations, 2 for subchronic to chronic extrapolation and a factor of 3 for data uncertainties at NOAEL level and incomplete bioaccumulation study data.

MTC_{tap} derivation:
- 60 kg bw per person, 2 l per day, 10 % allocation for drinking water

MTC_{tap} = 0.033 mg/kg bw/day * 60 kg bw / 2 l * 10 % = 0,1 mg/l

MTC_{tap} = 100 µg/l

8.9 Substance IX (CAS 6386-38-5)

Concentrations of substance IX at the tap may exceed 2.5 µg/l considerably.
Therefore, it is necessary to have access to oral dose toxicity data in addition to genotoxicity data.

8.9.1 Genotoxicity data

Bacterial reverse mutation assay

The test was carried out in 1983 with and without metabolic activation.

Table 20 Bacterial reverse mutation assay for substance IX

Test substance	
physical nature and purity	3-(3,5-Di-tert-Butyl-4-hydroxyphenyl)methylpropionate › 98%
stability of test substance	stable at room temperature
Solvent/Vehicle	
justification for choice of solvent	Acetone was chosen because of solubility of test substance
solubility/stability	Almost unsoluble in water
Strains	
straines used	TA 98, TA 100, TA 1535, TA 1537, TA 1538
Test conditions	
amount of test substance per plate	20, 80, 320, 1280, 5120 µg/0.1 ml
metabolic activation system	Aroclor induced rat liver cell S9-mix
Results	
	• Precipitation at 5120 µg/0.1 ml • positive and negative controls were carried out • no relevant increase of the revertants with and without metabolic activation

Conclusion: There is no evidence for mutagenicity from this test.

***in vitro* Chromosomal aberration on Chinese hamster ovary cells (OECD 473)**

The test was carried out in 1994 according to OECD-Guideline 473 and GLP. Two independent tests were carried out, each with and without metabolic activation.

Table 21 *In vitro* chromosomal aberration test for substance IX

Test substance	
physical nature and purity	3-(3,5-Di-tert-Butyl-4-hydroxyphenyl)methyl propionate › 98%
stability of test substance	stable at room temperature
Solvent/Vehicle	
justification for choice of solvent	Dimethylsulfoxide was the solvent, but there is no justification given
solubility/stability	no data given
Cells	
cells used	CHO CCL 61
Test conditions	
amount of test substance per plate	Exp. 1: 0, 4.88, 9.77 and 19.53 µg/ml (- S9) (18 h exposure)

	Exp. 2: 0, 4.88, 9.77 and 19.53 µg/ml (- S9) (42 h exposure) Exp. 3: 0, 39.06, 78.13 and 156.25 µg/ml (+S9) (3 h exposure, 15h recovery) EXP. 4: 0, 39.06, 78.13 and 156.25 µg/ml (+S9) (3 h exposure, 39h recovery)
metabolic activation system	Aroclor induced rat liver cell S9-mix
Harvest time after treatment	See above
Results	
	• Cytotoxic concentration at › 156.25 µg/ml (+S9) and › 19.53 µg/ml (-S9) • no relevant increase of chromosome aberration rate with and without metabolic activation • negative controls/solvent showed no increase of chromosome aberration rate • positive controls showed distinct increase of chromosome aberration rate • controls are in the range of historical controls

Conclusion: There is no evidence for clastogenicity from this test.

Source for both bacterial reverse mutation assay and chromosomal aberration studies: OECD-SIDS for Metilox/Arvin IX, section 5.5 "Genetic Toxicity In Vitro", pp. 59-60; references 4 and 18, respectively, cited therein.

On the basis of the abovementioned study data substance IX is assessed as not genotoxic with regard to possible mutagenicity and clastogenicity. Even though an *in vitro* mammalian cell micronucleus assay according to OECD TG 487 was not available for substance IX, the potential for aneugenicity can be excluded with high probability too, as the *in vitro* mammalian chromosomal aberration test according to OECD TG 473 showed no significant polyploidy above the solvent controls, which would be an indication for an aneugenic potential (also holds for substance X, see below).

8.9.2 Oral toxicity data

Developmental-/Reprotox Screening study

The test was carried out in 1994/1996 according to OECD 421 and GLP.
Source: Study Number 944072, "TK 10027 (Metilox): Reproduction/Developmental Toxicity Screening Test", CIBA-GEIGY Limited.

Table 22 Developmental-/Reprotox Screening study for substance IX

Test substance	
physical nature and purity	3-(3,5-Di-tert-Butyl-4-hydroxyphenyl)methyl propionate, ›98%
stability of test substance	stable at room temperature
Solvent/Vehicle	
justification for choice of solvent	0.5 % (w/w) aqueous solution of carboxymethyl cellulose in 0.1 % (w/w) Tween 80
solubility/stability	No data given
Test animals	
animals used	Rats (Tif: RAI f (SPF), hybrids of RII/1 x RII/2)
age	15-16 weeks (male) 10-11 weeks (female)
acclimatisation	1 week
Body weight	400-508 g male 207-269 g female
Number and sex	15 animals per sex and dose group
Test conditions	
dose level	0, 10, 100, 250 mg/kg/d
route, duration of administration and sampling	Intragastrically by gavage, 2 weeks before mating, throughout mating gestation and 4 days post partum. Males two weeks before mating and, during mating period and approx. 2 weeks after mating.
Results	
Mortality and clinical signs of parenteral animals	• In control group, one male died attributed to intubation error • In the 100 mg/kg group, one female died. At necropsy, mottled kidneys were observed. Doesn't indicate an association with the administration of the test substance. • In group 4 (250 mg/kg), nine males and 12 females displayed discomfort after test article application (pushing head through bedding) only for one to four days and was considered to be treatment-related. • Incidental clinical signs observed occasionally among surviving animals included palpable mass, hair loss, wound and/or crust/scurf, piloerection and chromodacryorrhea with no apparent relationship to treatment.
Body weight parenteral animals	• Body weights and food consumption were reduced at 250 mg/kg in males during the first week of the treatment.

	• In females at 250 and100 mg/kg, these parameters were reduced during premating, gestation and lactation
Reproductive function, Fertility	• Unimpaired
Litter indices	• Litter size at birth was slightly reduced at 250 mg/kg. In the same dose group, pup viability on day 4 post partum and mean pup weights were reduced.
Organ weight	• Mean absolute and relative liver weights were increased at 100 and 250 mg/kg (males and females).
Histopatholgy	• Increased incidence of hypertrophy of the hepatocytes in both sexes at 100 and 250 mg/kg. In the males, this change was accompanied by cytoplasmic inclusion bodies and was more pronounced than in the females. • Increased incidence of cholangiofibrosis of the intrahepatic bile ducts for both sexes at 250 mg/kg and for males at 100 mg/kg. • Higher incidences of inflammatory changes in the liver for both sexes at 100 and 250 mg/kg.

Conclusion: No observed adverse effect level given as 10 mg/kg body weight.

A 90 day feeding study reported in the REACH registration dossier gave a LOAEL of 70 mg/kg·d. The study is very old and was not conducted according to any harmonized protocols. Additionally, the rats were very young.

A screening test for reproduction and developmental toxicity was performed according to OECD guideline 421. 15 rats per dose and sex were administered to 0, 10, 100 and 250 mg/kg·d by gavage. A NOAEL of 10 mg/kg·d for parental toxicity was determined due to reduced food consumption, reduced body weight gain, hypertrophy of liver hepatocytes. A NOAEL for developmental toxicity was determined at 100 mg/kg·d due to reduced litter size at 250 mg/kg·d as well as reduced pup weights and pup viability.

8.9.3 MTC_{tap} calculation

As substance IX hydrolyses very fast at pH 7.4 in rat serum und liver homogenizate to the corresponding acid (substance X), an MTC_{tap} should be derived for both substances together. As the abovementioned oral toxicity data would result in higher MTC_{tap} than the oral toxicity data given for substance X, the starting point for a MTC_{tap} calculation is settled in the oral toxicity data of substance X; see below.

8.10 Substance X (CAS 20170-32-5)

8.10.1 Genotoxicity data

Bacterial reverse mutation assay

The test was carried out in 1983 with and without metabolic activation in two independent tests.
Source: "Salmonella / mammalian microsome mutagenicity test", Test material: TK 10 797 (Metiloxcarbonsäure), Project No.: 830492, CIBA-GEIGY Limited.

Table 18 Bacterial reverse mutation assay for substance X

Test substance	
physical nature and purity	3-(3,5-Di-tert-Butyl-4-hydroxyphenyl)methylpropionic acid › 98.5%
stability of test substance	stable at room temperature
Solvent/Vehicle	
justification for choice of solvent	Acetone was chosen because of solubility of test substance
solubility/stability	No data given
Strains	
straines used	TA 98, TA 100, TA 1535, TA 1537, TA 1538
Test conditions	
amount of test substance per plate	20, 80, 320, 1280, 5120 µg/0.1 ml Tests performed in triplicate
metabolic activation system	Aroclor induced rat liver cell S9-mix
Results	
	• Precipitation at 1280 µg/0.1 ml • positive and negative controls were carried out • no relevant increase of the revertants with and without metabolic activation

Conclusion: There is no evidence for mutagenicity from this test.

***in vitro* Chromosomal aberration on Chinese hamster ovary cells (OECD 473)**

The test was carried out in 1992 according to OECD-Guideline 473 and GLP. Two independent tests were carried out, each with and without metabolic activation.
Source: "Cytogenetic test on Chinese hamster cells *in vitro*", Test number: 924153, Test substance: TK 10 797 [Metilox carbonic acid], CIBA-GEIGY Limited.

Table 19 *In vitro* chromosomal aberration test for substance X

Test substance	
physical nature and purity	3-(3,5-Di-tert-Butyl-4-hydroxyphenyl)methylpropionic acid 99.52 %
stability of test substance	stable at room temperature
Solvent/Vehicle	
justification for choice of solvent	Dimethylsulfoxide was the solvent, but there is no justification given
solubility/stability	no data given
Cells	
cells used	CHO CCL 61
Test conditions	
amount of test substance per plate	Exp. 1: 0, 51.88, 103.75 and 207.5 µg/ml (- S9) (18 h exposure) Exp. 2: 0, 25.94, 51.88 and 103.75 µg/ml (- S9) (42 h exposure) Exp. 3: 0, 103.75, 207.5 and 415.0 µg/ml (+S9) (3 h exposure, 15h recovery) EXP. 4: 0, 103.75, 207.5 and 415.0 µg/ml (+S9) (3 h exposure, 39h recovery)
metabolic activation system	Aroclor induced rat liver cell S9-mix
Harvest time after treatment	See above
Results	
	• Cytotoxic concentration at › 103.75 µg/ml/ › 207.5 µg/ml (+S9) and › 415.0 µg/ml (-S9) • no relevant increase of chromosome aberration rate with and without metabolic activation • negative controls/solvent showed no increase of chromosome aberration rate • positive controls showed distinct increase of chromosome aberration rate • controls are in the range of historical controls

Conclusion: There is no evidence for clastogenicity from this test.

On the basis of the abovementioned studies substance X is assessed as not genotoxic with regard to possible mutagenicity and clastogenicity.
As already stated in the preceding section on substance IX, even though an *in vitro* mammalian cell micronucleus assay according to OECD TG 487 was not available, the potential for aneugenicity can be excluded with high probability for substance X too, as the *in vitro* mammalian chromosomal aberration test according to OECD TG 473 for substance X showed no significant polyploidy above the solvent controls.

8.10.2 Oral toxicity data

Subchronic oral toxicity study

The test was carried out in 1973.
Source: "13-week oral toxicity study in rats with compound TK 10 797" [Metilox]; CIBA-GEIGY Limited

Table 20 13-week oral study for substance X

Test substance	
physical nature and purity	3-(3,5-Di-tert-Butyl-4-hydroxyphenyl)propionic acid, no data on purity
stability of test substance	no data given
Solvent/Vehicle	
justification for choice of solvent	1 % (w/w) Gum acacia
solubility/stability	No data given
Test animals	
animals used	Sprague Dawley rats
age	no data given
acclimatisation	no data given
Body weight	113 g male 109 g female
Number and sex	15 animals per sex and dose group
Test conditions	
dose level	0, 50, 150, 500 mg/kg/d
route, duration of administration and sampling	by gavage, 13 weeks.
Results	
Mortality and clinical signs	• at dose level 500 mg/kg 22 animals died • at dose level 500 mg/kg loss of physical condition, thyroid hyperplasia, liver necrosis and hypertrophy occurred • at dose level 150 mg/kg one animal died, the cause was not ascertained • at dose level 150 mg/kg thyroid hyperplasia in both sexes, liver changes as reported for 500 mg/kg but without necrosis occurred in male rats only • at dose level 50 mg/kg only liver hypertrophy relevant to treatment

Conclusion: No observed adverse effect level given as 50 mg/kg body weight.

8.10.3 Oral toxicity data

90-day oral study

The test was carried out in 1983 according to OECD 408 and GLP.
Source: TK 10797 – "3 month oral toxicity study in rats" – GU Project No. 810895, CIBA-GEIGY Ltd.

Table 21 90-day oral study for substance X

Test substance	
physical nature and purity	3-(3,5-Di-tert-Butyl-4-hydroxyphenyl)propionic acid, 98.5%
stability of test substance	stable at room temperature
Solvent/Vehicle	
justification for choice of solvent	0.5 % (w/w) aqueous solution of carboxymethyl cellulose in 0.1 % (w/w) Tween 80
solubility/stability	stability was tested after 4 hours room temperature
Test animals	
animals used	Rats (Tif: RAI f (SPF), hybrids of RII/1 x RII/2)
age	6 weeks
acclimatisation	no data given
Body weight	165-169 g male 135-141 g female
Number and sex	20 animals per sex and dose group
Test conditions	
dose level	0, 3, 10, 30, 100 mg/kg/d
route, duration of administration and sampling	Intragastrically by gavage, 3 months
Results	
Mortality and clinical signs	• 3 animals died, two of them because of misapplication and on (dose group 100 mg/kg) with no macroscopial findings • no clinical signs of local and systemic toxicity in treated animals
Body weight	• Body weight gain of all animals similar to control group • Food consumption of all treated animals similar to control group, slight trend in increased water consumption for animals of highest dose group
Organ weight	• liver weights increased in dose groups 10, 30 and 100 mg/kg

	• slight increase of adrenals weight in dose group 30 and 100 mg/kg
Histopatholgy	• Increased incidence of hypertrophy of the hepatocytes at 30 and 100 mg/kg. • focal accumulation of foamy cells in the alveoli in dose group 100 mg/kg

Conclusion: No observed effect level given as 3 mg/kg body weight.

In a 13-weeks oral study 15 rats per sex and dose group were administered to 0, 50, 150 and 500 mg/kg · d by gavage. In the highest dose group reduced body weight gain and food consumption as well as reduced physical condition occurred. Additionally, thyroid hyperplasia and increased liver weights with necrosis and hypertrophy could be recognized. Small droplets of fat were present in the proximal convoluted epithelium of the kidneys. Besides reduced haemoglobin, haematocrit and erythrocyte values were seen in the blood samples. In the 150 mg/kg · d dose group reduced body weight gain, enlarged thyroids and increased liver and kidney weights occurred. Thyroid hyperplasia were observed.
The 50 mg/kg · d dose group showed no treatment related effects and could be seen as NOAEL.

In a 90-day oral study 20 rats per sex and dose were administered to 0, 3, 10, 30 and 100 mg/kg · d by gavage. No treatment related mortality or effects on body weight gain and food consumption occurred. There were no clinical signs and no toxicity observed. The examined blood parameters were in the typical physiological range. Increased liver weights could be observed in the dose groups of 10, 30 and 100 mg/kg · d which can be regarded as adaptive due to lacking histopathological or biochemical effects. A slight increase of adrenals weight was observed in dose groups 30 and 100 mg/kg · d. Histopathology showed hypertrophy of hepatocytes in the dose groups 30 and 100 mg/kg · d and focal accumulation of foamy cells in the alveoli in the highest dose group.
The 3 mg/kg · d dose group is considered as NOEL (no observed effect level) and 10 mg/kg · d is considered as NOAEL (no observed adverse effect level).

Toxicokinetics and possible bioaccumulation of Arvin X

The above 90-day oral toxicity study applying daily administration of substance X, though not monitoring substance accumulation and elimination but an array of behavioural, clinical and organ parameters, allowed for derivation of a NOAEL from absence of adverse effects below the dosing range of 10 mg/kg · d.

To account for a possible bioaccumulation potential of the substance, a single-dose oral 14-day toxicokinetic study performed in 1990 was evaluated, applying ^{14}C-labeled target substance and activity counting for fate and recovery monitoring (source: “TK 10797: Absorption, Distribution and Excretion after Single Oral Administration to the Rat”. RCC Umweltchemie AG, Itingen, Switzerland. Project-no. 894422. August 29, 1990). In this study, male rats in groups of 5 individuals were administered a single dose of 1 and 10 mg/kg body weight of ^{14}C-labelled compound. Substance fate was monitored by radioactivity analysis

(activity counting) in faeces and urine over a period of 168 hours and residual radioactivity in tissues and organs. Excretion after 168 hours via urine and faeces amounted to between 46 and 43 % and between 49 and 55 %, respectively, depending on dose levels low and high. More than 90 % of absorbed substance was excreted after 48h. Residual activity in tissues/organs was low. Maximum plasma levels were reached already after one hour, indicating rapid absorption. Elimination half-times ranged from 3.0 to 10.4 hours. In conclusion, no indication for accumulation in animals was observed.

In general, a study exercising repeated dosing over a 14 days period is recommendable because histological changes in liver, kidney and other organs have been observed and repeated exposure is associated with drinking water consumption. However, in view of indications from the 90-day oral toxicity and lacking evidence for possible relevant substance accumulation from the single dose 14-day toxicokinetic study, it seems inadequate to request for a repeated dose toxicokinetic study. Currently, UBA cannot perform read-across dealing with comparable relevant substances due to lack of existing data.

In order to account for drawbacks of the existing single dose toxicokinetic study and to achieve a reasonable evaluation, an additional uncertainty factor is introduced. To account for uncertainties of possible accumulation in man, a factor of 10 is generally applied. In the present case, regarding the existing toxicokinetic study (no evidence for accumulation from single administration) and the existing 90 days study this uncertainty factor can be reduced to 3.
From the proposed NOAEL of 10 mg/kg b.w., the safety factors for inter- and intraspecies variability amounting to 100, the extrapolation factor from subchronic to chronic exposure amounting to 2 and the above uncertainty factor for possible accumulation amounting to 3, an overall safety factor of 600 results.

8.10.4 MTC_{tap} calculation

The 90-day oral study gave a NOEL of 3 mg/kg · d. In the next higher dose group of 10 mg/kg · d increased liver weights occurred but without histopathological or biochemical effects. Therefore, the increased liver weights could be seen as adaptive and 10 mg/kg · d as NOAEL (no observed adverse effect level).
Starting from this value, applying a factor of 100 for inter- and intra-species derivation, an uncertainty factor of 2 (90 day instead of lifetime) and an additional uncertainty factor of 3 for possible accumulation results in a TDI analogous value of 16.7 µg/kg · d.
Assuming a body weight of 60 kg, a consumption of 2 liters of water per day and an allocation factor of 10 % for drinking water results in a MTC_{tap} of **50 µg/l**.

This MTC_{tap} is applicable as total value (sum) for substance IX and X because from metabolization studies it is known that at pH 7.4 in rat serum and liver homogenate substance IX hydrolyses very fast to the corresponding propionic acid (substance X).

9 Summarized conclusion for stabilizer degradation product substances

Substance II has already been assessed for food contact materials in Regulation (EU) No. 10/2011 with an SML of 50 mg/kg. This results in a MTC_{tap} of 2.5 µg/l.
There were no data available for substance V. Therefore, a MTC_{tap} of 0.1 µg/l would apply.

Results of genotoxicity tests were available for substances I, III, IV, VI, VII, VIII, IX and X and for none of them showed evidence of genotoxicity. Additionally, oral studies were available for substances IV, VIII, IX and X.

The following MTC_{tap} result for individual substances considered here and are compared to the migration results already mentioned (table 5):

Table 22 MTC_{tap} for individual stabilizer degradation product substances

Substance No.	Name	CAS	MTC_{tap} [µg/l]	UBA (GC/MS-screening)	
				C_{tap} [µg/l], 23°C	C_{tap} [µg/l], 60°C
I	4-Ethylphenol	123-07-9	0.1	n.d.	n.d.
II	p-tert-Butylphenol	98-54-4	2.5	0.1	0.1 – 0.2
III	2,6-Di-tert-butyl-p-benzoquinone	719-22-2	2.5	0.02 – 0.6	0.16 – 4.8
IV	2,4-Di-tert-butylphenol	96-76-4	250	0.3 – 6.2	0.46 – 28.8
V	3,5-Di-tert-butyl-4-hydroxystyrene	19263-36-6	0.1	n.d.	n.d.
VI	3,5-Di-tert-butyl-4-hydroxybenzaldehyde	1620-98-0	2.5	0.01 – 1.7	0.1 – 3.8
VII	3,5-Di-tert-butyl-4-hydroxyacetophenone	14035-33-7	2.5	n.d.	n.d.
VIII	7,9-Di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	82304-66-3	100	0.04 – 2.9	1.7 – 49.8
IX	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)methyl propionate	6386-38-5	50 (∑ IX+X)	0.1 – 4.1	0.1 – 29.2
X	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propionic acid	20170-32-5		n.d.	0.17 – 17.7

STATEMENT

In accordance with the 4MSI approach, Germany recommends that migration limits for degradation product substances from listed stabilizers will be set as additional requirements for plastics in the 4MSI Common Approach on Organic Materials in Contact with Drinking Water Part C - Procedures and Methods for Testing and Accepting Products Made of Organic Materials as follows:

Substance No.	Name	CAS	MTC_{tap} [µg/l]
I	4-Ethylphenol	123-07-9	0.1
II	p-tert-Butylphenol	98-54-4	2.5
III	2,6-Di-tert-butyl-p-benzoquinone	719-22-2	2.5
IV	2,4-Di-tert-butylphenol	96-76-4	250
V	3,5-Di-tert-butyl-4-hydroxystyrene	19263-36-6	0.1
VI	3,5-Di-tert-butyl-4-hydroxybenzaldehyde	1620-98-0	2.5
VII	3,5-Di-tert-butyl-4-hydroxyacetophenone	14035-33-7	2.5
VIII	7,9-Di-tert-butyl-1-oxaspiro[4.5] deca-6,9-diene-2,8-dione	82304-66-3	100
IX	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)methyl propionate	6386-38-5	50 (∑ IX+X)
X	3-(3,5-Di-tert-butyl-4-hydroxyphenyl)propionic acid	20170-32-5	