# Precautionary Risk Assessment and Risk Management of Chemicals

Part II: Chemicals in the Environment which Interfere with the Endocrine Systems of Humans and Wildlife

# Chemicals in the Environment which Enterfere with the Endocrine Systems of Humans and Wildlife

## – Pollution, Effects, Control Strategies –

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# 1. Introduction

In 1995, the Federal Environmental Agency (UBA) hosted the first symposium on endocrine disrupters in the environment (UBA-Texte 65/95). Since them, an intense debate has been conducted in Germany on the related mechanisms, pollution levels and resulting risks to public health and the environment. Opinions on these substances and their endocrine effects are still strongly divided, not only among the various interest groups, but also among experts themselves. An international register of current research projects in this field shows Germany to be the most active country in Europe, with 50 current projects. Both the intensive research and the debate on risk assessment have meant that – despite the large gaps in our knowledge which remain – the importance of this problem in Germany can be assessed in far greater detail than ever before.

In addition, the European Parliament, European Commission and many national governments in the EU have come to recognise the importance the issue of endocrine disrupters, and are asking for more activity with a greater integration into the chemicals safety programme. A key document is the Commission's 17/12/1999 communication on a community strategy for endocrine disrupters (COM 99/706), which sets out the necessity of further research, informing the public and taking political action. Short-term proposals are prioritising the various substances, primarily with respect to the risks associated with their hormonal effects, applying existing legal regulations (e.g. assessing high-priority substances according to the Existing Substances Regulation (EC 793/93)) and deciding on monitoring programmes, international co-ordination and information for the general public. In the medium term, xenobiotic endocrine disrupters should be determined and assessed, and impetus given to research and development into improved evaluation of the consequences. This has already begun. In the long term, the EU legal framework on chemicals, crop protection agents and biocides may require adjustment.

In August 1999 a government decision in the German Bundestag called for a staged but drastic reduction in discharges of proven endocrine disrupters (14/1471 of 4/8/2000), drawing on the similar decision by the European parliament on 26th January 1999. Furthermore, it asked that those chemicals which can also reach ground water and drinking supplies, and which can regularly be shown to have done so, should be banned, and limits for drinking water should be determined. The use of environmental chemicals should also be reduced where there is reason to suspect that they are endocrine disrupters. Domestically, special measures should be taken for alkylphenol(ethoxylate)s, phthalates and tributyltin compounds. In a decision on 26$^{th}$ October 2000, the EU parliament once again called upon the

Commission, in the strongest terms, to take rapid action to reduce the risks from endocrine disrupters, rather than waiting for further tests.

The greater emphasis on precaution, expressed in e.g. the EU Commission's 2/2/00 white paper on the precautionary principle (COM 2000 (1)), makes it necessary to examine whether our current state of knowledge about environmental chemicals which disrupt the endocrine system calls for precautionary reduction measures.

In this context, the following report

- assesses existing epidemiological knowledge on detriments to human health,
- briefly outlines the levels of pollution by important endocrine disrupters in the environmental media,
- describes the current state of development in procedures for testing the endocrine effects ,
- lists the environmental chemicals currently considered by the EU to be endocrine disrupters, and assesses the need for regulation
- proposes measures to influence public behaviour directly and indirectly, improve our knowledge and reduce risks.

## 2. Assessing existing knowledge on detriments to human health

It is undisputed that a number of substances are able to disrupt endocrine processes, with the potential for impairing development and reproduction or increasing the risk of cancer[1]. However, to fully evaluate the risks, we must assess the probability that biologically significant concentrations of such substances may be present now or in the past in foods, drinking water or environmental compartments, i.e. whether they could, under realistic conditions, in fact trigger harmful effects in humans and animals. To answer this question, information on a potential endocrine disrupter must be available, both as to its activity, i.e. dependency on dosage or concentrations, and as to the actual concentrations, i.e. human and animal exposure.

However, even today there are no reliable data (which can be extended to different animal species or humans) on the potential of known endocrine disrupters for triggering significant effects. Results from *in vitro* testing of isolated hormone receptors or cell cultures, as are available for many xeno-estrogens, are inadequate for a number of reasons.[2]

It is therefore surprising that in 1999 the BUA (GDCh-Advisory Committee on Existing Chemicals) and subsequently the German Council of Environmental Advisors (in its 1999

special report on environment and health) expressed the view that "the significance of endocrine disruptors for human health has been exaggerated"[3].

The following details the important aspects in assessing significant harm to humans associated with the effects of endocrine disrupters.

### *2.1 Male reproductive functions*

#### *2.1.1 Sperm quality*

The debate on the possibility of human health being harmed by substances with effects on the endocrine system was initiated by a supposed reduction in mens' sperm quality in industrialised countries. A meta-analysis by Carlsen et al.[4] originally concluded that the sperm count in the ejaculate from test subjects has fallen by c. 50% between 1938 and 1990. There followed a detailed discussion in the scientific literature[5] as to the significance of the results and the likelihood that environmental pollutants had played a role in this development. Taking this criticism into account Swan et al.[6] reevaluated the data, confirming a fall in sperm count in western Europe (but not elsewhere) and excluding the possibility of statistical errors in the sampling and analysis. As the authors nonetheless admitted, "We have not addressed the cause(s) of this decline or assumed an environmental aetiology", stating in later work: "Although few of these trend studies have examined possible causes, common environmental exposures are plausible."[7] Other writers studying the phenomenon of falling sperm counts also discuss cultural, socio-economic as well as environmental factors as potential causes. A study co-ordinated between Finland, Scotland, Denmark, France and Japan has now shown that sperm quality varies geographically[8,9], making the findings here especially important in assessing the situation for Germany. There are now three studies in Germany, examining sperm quality trends in thousands of test subjects from fertility advice centres. The tests in Hamburg[10], Leipzig[11] and Magdeburg[12] revealed significant falls in sperm quality parameters, shown relative to Swan's calculations for western Europe in Fig. 1.

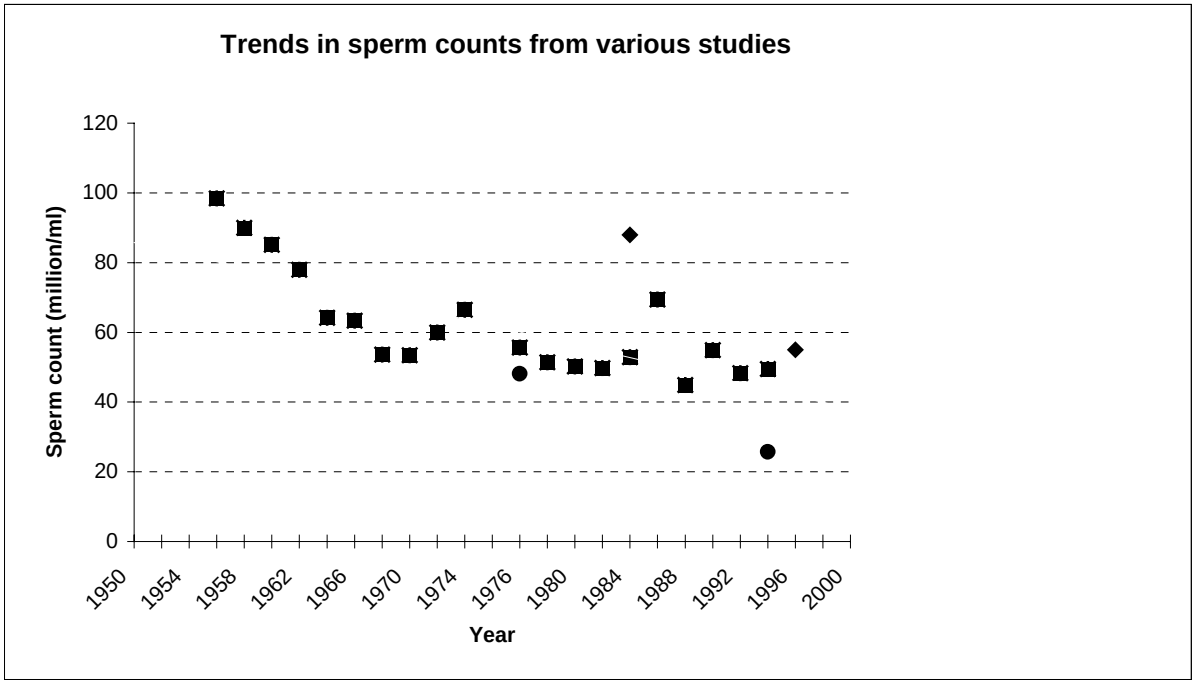


**Figure 1:** Linearised graph of trends in sperm counts from various German studies. Sources: see text.

In interpreting these observed results, the following should be noted:

- The studies each comprised several thousand test subjects. They are among the largest in the world, and can be considered correspondingly reliable. The men tested had sought advice on fertility problems, and are therefore not typical for the entire population. Nonetheless, similarly large-scale surveys in France, for example, where the sample was more representative of the population as a whole, show similar results[13]. In Thierfelder's study at least, the sperm characteristics of men where no results were found relating to the causes of their infertility were considered separately.

- The fall in sperm quality can be found in both of the former German states, but appears to set in earlier in the west than in the east. For the past 10 years, the data from Hamburg show stabilisation at a low concentration, around the 20 mil./ml described by the WHO as critical for fertility. A new study of not preselected recruits in Denmark[14], shows similar concentrations to those in Hamburg. This also implies that the data collected from German men with fertility problems are indicative for the entire population.

- The reduction of sperm counts appears to depend more on date of birth than on the date the tests were made. This means that, surprisingly, older men have better sperm counts than younger men, making it likely that the damage occurs before birth or during development.

- Wearing tight trousers, or eating soya-based meals rich in phytoestrogens, both proposed explanations - which were not substantiated by studies[3] - appear less able to explain the observed phenomena[3], as these lifestyle factors did not apply in the former GDR. However, other - equally controversial - possible causes are being considered: consumption of alcohol, cigarettes, caffeine, etc. (which were equally prevalent in the GDR).

- The geographical variance observed in western countries could imply the influence of still unknown lifestyle or environmental factors.

- This raises the question of how far falling sperm counts affect the fertility of the German male population. WHO guidelines define a sperm count below 20 mil./ml as abnormal[15], a significant reduction on the previous norm[16] of 40 mil./ml. Danish tests on the connection between sperm count and fertility have shown that male fertility is impaired if the sperm count is below 40 mil./ml.[17]

- Overall, the hypothesis that the cause lies in chemical effects has become increasingly likely.

**Assessment:**
***Are there changes in sperm quality in Germany which could be caused by environmental factors?***
Yes. Several independent studies have found a significant deterioration in the quality of sperm in men from western and eastern Germany.
***Is there evidence that similar phenomena have occurred in the sons of women who took DES (diethylstilbestrol), an artificial estrogen, during pregnancy?***
Yes. Several studies have found sperm counts about one third lower than in the control population in the sons of women treated with DES[18].
***Have epidemiological studies or animal experiments provided evidence that the development could have been caused by phytoestrogens?***
No.[19]
***Have epidemiological studies or animal experiments provided evidence that the development could have been caused by industrial chemicals or pesticides?***
Yes. An animal experiment has shown that low doses of xeno-estrogens (e.g. bisphenol A[20]) and antiandrogens (e.g. dibutylphthalate[21]) disrupt sperm production, although the results and design of the experiment are a matter of heated discussion.

*2.1.2 Testicular cancer*

The incidence of testicular cancer standardised for age is obviously rising continuously. Although testicular cancer is still not a frequent form of cancer, it can occur in younger men, which gives it a high importance for society. It is supposed that lifestyle and environmental factors, as well as genetic predisposition and workplace conditions, play a role in the development of testicular cancer. This is indicated by the increased incidences and the significant geographical variance[22]. In Denmark, for example, the incidence rose by c. 2.6% annually between 1943 and 1996. However, the increase has tended to fall recently (since c. 1985), especially in men born after 1963. In the USA, however, the incidence of testicular tumours has risen in white, but not black men. This implies a significance for genetic predisposition.[23]

The development of sexual organs is hormone-dependent, and it is therefore plausible that affecting the hormonal influence on this development could also affect the development of testicular cancer. There is no experimental proof, as there is no suitable animal model for the most frequent form of testicular cancer in men (seminoma). On the other hand, a meta-analysis by Toppari et al.[24], implies that prenatal exposure to therapeutic DES is a significant risk factor for testicular cancer: the risk of testicular cancer was 2.6 times higher for the sons of women who were treated with DES than for the population as a whole.

Recent studies, such as those by Moller[25] and Jacobsen[26], suggest a common aetiology for deteriorating sperm quality and the risk of testicular cancer. The authors present the following evidence:

- Men who have fathered children are at a significantly lower risk of developing testicular cancer.
- Men with poor sperm characteristics have a higher risk of testicular cancer.
- Men with a low relative fertility (i.e. who have fathered less than the average number of children for their age group) have twice the risk of getting testicular cancer.
- However: while low relative fertility can be related to testicular cancer, the cancer risk is not lower for men with above-average fertility.
- Men in marriages with fertility problems have a high probability of developing testicular cancer.
- Low sperm count and mobility, and an increased level of abnormally shaped spermatozoa correlate to an increased risk of testicular cancer.

In the view of this report, these data are consistent with the hypothesis that male subfertility and poor sperm have aetiological factors in common with testicular cancer.

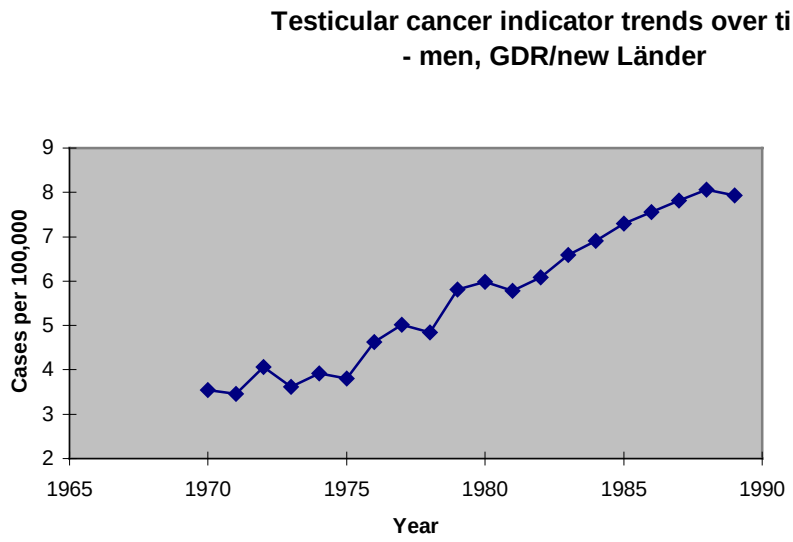


**Figure 2**: Incidence of testicular cancer in the GDR, grouped by age.

Source: Cancer Atlas of the GDR[27]

The aforementioned pilot study[22] traces the incidence of testicular cancer in the GDR between 1961 and 1989 on the basis of the "Cancer Atlas of the GDR". The incidence, grouped by age, quadrupled steadily over the 28 years, from 2 to 8 cases per 100,000, an annual increase of 5%. Urban districts were found to have a 25% higher risk of illness than rural districts.

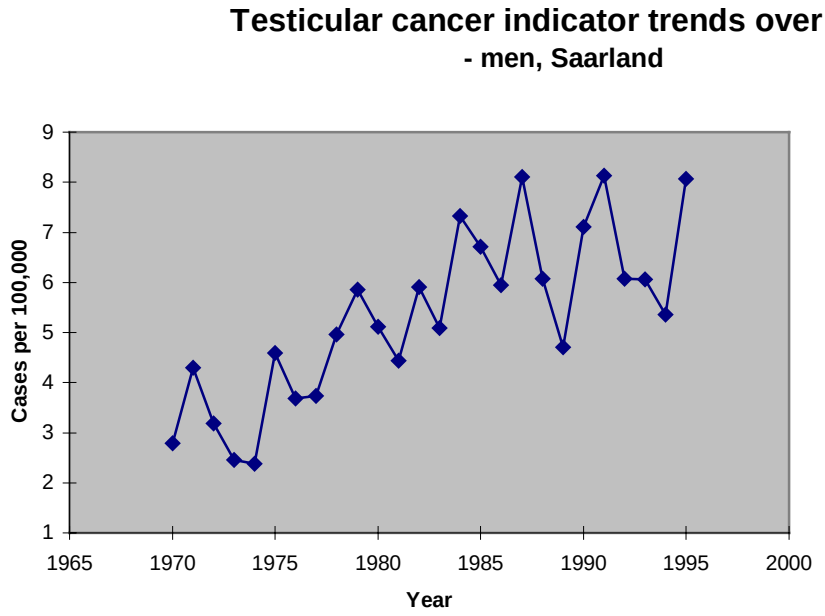


**Figure 3:** Incidence of testicular cancer in Saarland, 1970-1995, grouped by age. Source RKI

The Saarland cancer register shows an increase in new cases of testicular cancer from 2.8 to 8.1 cases per 100,000 between 1970 and 1995.

**Assessment:**
As in most industrialised countries, Germany is also experiencing an increase in new cases of testicular cancer cases. Recent studies suggest that deteriorating sperm quality and increased testicular cancer could have a common aetiology. The relevance of estrogenic substances appears plausible, due to the increased risk of testicular cancer found in men who had been exposed to DES *in utero*. However, the lack of prospective studies, the long latency period of the illness after prenatal exposure and the lack of animal models for this form of cancer make it impossible to prove causality.

*2.1.3 Prostate cancer*

The incidence of prostate cancer in the GDR doubled between 1961 and 1989, from 12 to 24 cases per 100,000. In Saarland, the rate of new cases per 100,000 rose from 33 to 62.

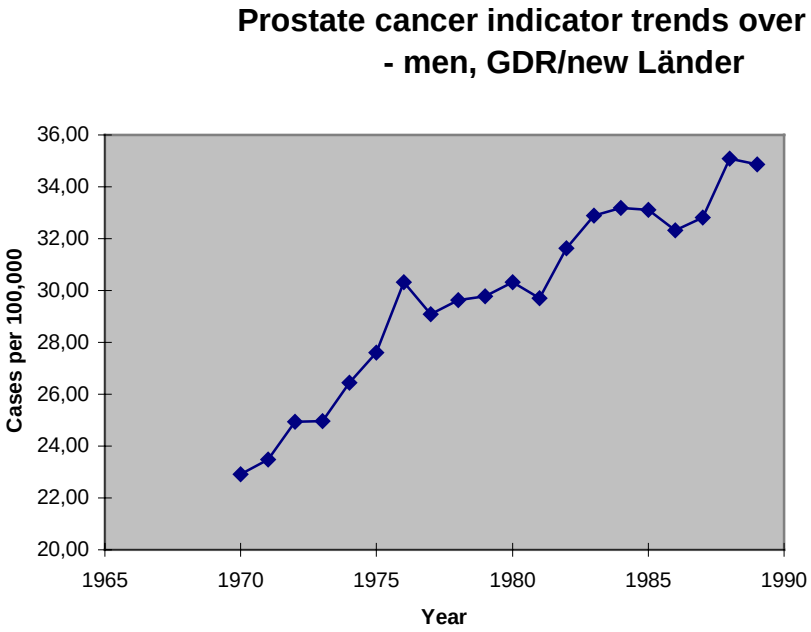


**Figure 4**: Incidence of prostate cancer in the GDR, 1961-1989, grouped by age
Source: Cancer Atlas of the GDR[27]

The increased incidence is also confirmed by the other German cancer registers[28]. Increases in incidence and mortality can be found internationally for the illness, although there is very great variance in the rates[29]. Regions where there is a high incidence of prostate cancer in men correlate with high levels of breast cancer in women[30]. Several epidemiological studies describe how users of pesticides have a significantly increased risk of developing prostate

cancer[31,32,33,34]. This can be partially traced back to the endocrine effects of pesticide use, although the actual pesticides are unknown[35].

There is also a connection between increased levels of estrogen in mothers during pregnancy and neoplastic development in the prostates of male children. Cellular change in the prostate during pre- and perinatal development can make these cells especially sensitive to the neoplastic effects of testosterone and estrogen later[36]. This would form a plausible explanation for a connection between xeno-estrogen and increased incidence of illness.

**Assessment:**
There is currently no evidence for a causal connection between exposure to endocrinally active substances and an increased incidence of prostate cancer. However, this increase could hypothetically be explained by the effects of endocrine disrupters.

*2.1.4 Malformation in male genitals*

A number of authors[24] has postulated a connection between the occurrence of genital malformation - in particular cryptorchism and hypospadias - and the prenatal exposure to endocrine disrupters. A Spanish epidemiological study points to a possible connection between pesticide use and the frequency of male genital malformation[37]. In order to clarify whether existing studies and observations in Germany exhibit a trend in the frequency of genital malformation over time, the UBA commissioned a pilot study to summarise and evaluate the data on the prevalence of genital malformation, and produce hypotheses as to the causes[22], the results of which are now available. Genital malformation has been selected as a symptom, as there is a short time span between possible external factors and the symptom, and therefore a correlation between exposure and its effects is most likely to be found.

However, the pilot study could prove no uniform trends in the incidence of genital malformation over time. The data collection methods within Germany and across Europe are not uniform and not suited to the issue under examination. The frequencies determined by the different institutions vary hugely. The study's authors plead for a uniform nation-wide register of malformations to register regional and temporal trends, and also point to the possibility of using existing precautionary examinations of children.

**Assessment:**
Currently available surveys do not permit a final judgement as to whether genital malformations are occurring more frequently in new-born males than earlier. As yet there is no malformation register to record these abnormalities uniformly and with the necessary precision. Such a register would be highly desirable, not least because connections between increased malformation and pollution are being made more and more often.

## *2.2 Female health*

### *2.2.1 Breast cancer*

Breast cancer is the most frequent form of cancer in women, and exposure to estrogen is one risk factor in its incidence. However, a distinction can be drawn between estrogen-sensitive breast tumours and others. According to Glass and Hoover[38], the incidence of estrogen-sensitive tumours has risen significantly faster in recent decades than that of other breast cancers. Since the publication of a study by Wolff in 1993[39], which linked levels of DDT and its metabolites in the body with the incidence of breast cancer, there has been a heated debate as to the significance of xeno-estrogens in the development of breast cancer. Years or decades normally pass between exposure and the incidence of the illness, and retrospective studies therefore have great difficulty in identifying a connection between exposure and increased incidence. Since personal factors, for example nutrition or hormone levels in various phases of life, also affect cancer development, designing suitable studies is even more difficult.

Neither the larger retrospective studies nor more recent prospective studies have been able to reinforce the suspected link between exposure to DDT and the frequency of breast cancer[40,41,42,43,44]. The same applies for studies of PCB and breast cancer.

Apart from these two groups, there have been few studies of the effects of endocrine disrupters on incidence of breast cancer. Two recent prospective studies from the USA[43] and Denmark[42] show that hexachlorobenzene and dieldrine may also be risk factors in the development of breast cancer.

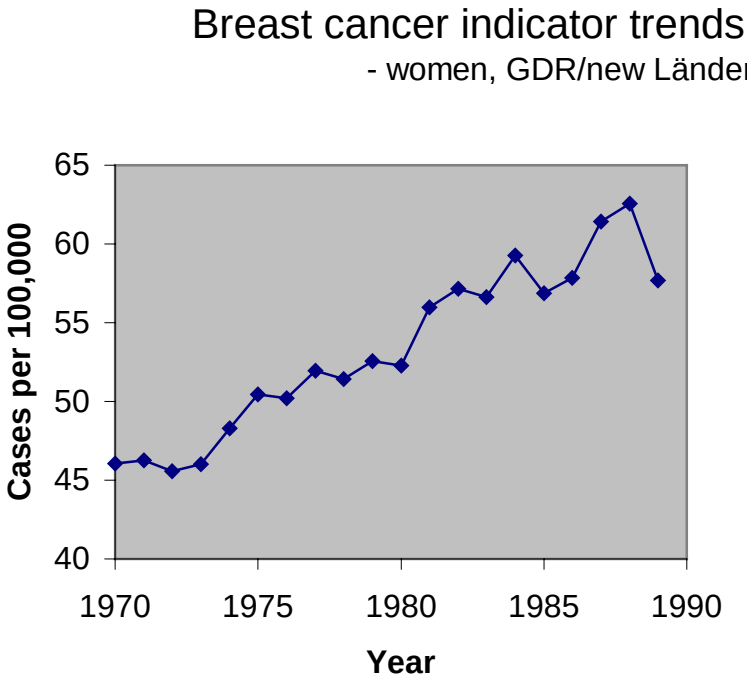


**Figure 5**: Incidence of breast cancer in the GDR, 1961-1989, grouped by age.

Source: Cancer Atlas of the GDR[27]

An upwards trend in new cases of breast cancer is emerging in Germany. In the former GDR, this rise was from 27 cases per 100,000 in 1991 to 45 in 1995.

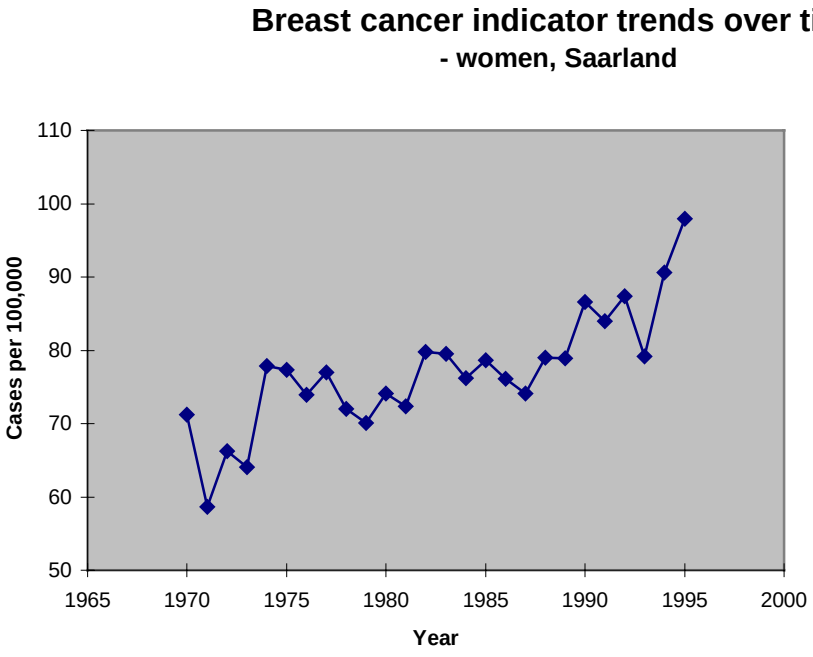


**Figure 6:** Incidence of breast cancer in Saarland, 1970-1995, grouped by age. Source RKI

The Saarland cancer register shows a rise from 71 cases per 100,000 in 1971 to 98 in 1997.

However, there is no disputing that factors such as genetic predisposition and western industrialised lifestyles (nutrition, tobacco and alcohol use and lack of physical activity) play a major role in the development of breast cancer. Other important factors are when menstruation and menopause begin, the number of children borne and at what age, as well as specific hormone treatments.

**Assessment:**
Studies to date have restricted themselves more or less exclusively to the links between DDT and breast cancer. According to the vast majority of the studies (in particular the better studies), there does not appear to be such a link. The discovery in recent prospective studies of positive links with other pesticides make further studies desirable.

*2.2.2 Early Puberty*

There are very few studies of the question as to whether an early start into puberty may be linked to exposure to chemicals. In an article in "Nature", Howdeshell et al. note that the estrogenic industrial chemical bisphenol A given in very low perinatal doses leads to an earlier occurrence of puberty in laboratory animals[45], although there is no precise description of the experiment. In Central Europe the menarche has been observed to occur on average three months earlier per decade.

***2.3 Other changes related to reproduction***

*2.3.1 Gender ratio*

The gender ratio in new borns is known to be 106 male to 100 female[46]. This ratio is maintained by hormone concentrations in the parents at the moment of conception. Changes in the levels of gonadotropine or steroids can produce a change in this ratio. It is known that men exposed to dioxins have a significantly lower level of testosterone and a higher level of gonadotropine.[47] Tests of the population affected by the accident at Seveso in 1976 have shown that females are significantly overrepresented in the offspring of those exposed to high levels of dioxins[48]. Similar shifts towards female births were observed where the fathers had been exposed to high doses of the antiandrogenic pesticide vinclozoline[49] or organo-chlorinated pesticides[50].

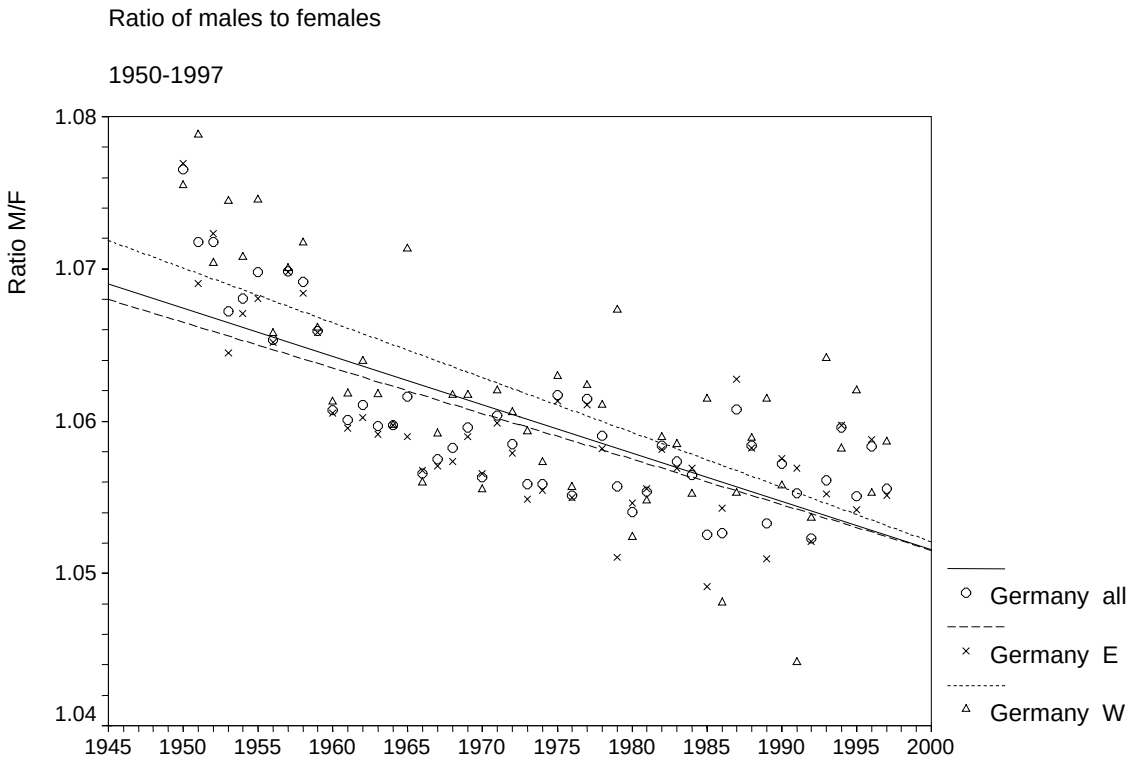


**Figure 7:** Gender ratio in Germany. Linearised trends in the whole of Germany, the new Länder and western Germany. Source: Rösch et al.[22]

A rising trend in the relative numbers of new-born females has been observed in many industrial countries[51], although geographical differences have been reported here too. Astolfi and Zonta have found a fall in male births in Italian conurbations, while on the other hand, more males are being born in rural areas. The Magdeburg pilot study has evaluated the statistics on the gender of new births.[22] A highly significant fall in the number of male births can be shown, both in Germany as a whole and in eastern or western Germany. Between 1950 and 1997, the ratio of male to female births dell from 1.08 to 1.05. This shift clearly began in the 1950s and is continuing. Vartiainen et al. have studied live births in Finland since 1751. While there was an increase in the proportion of male births until 1920, there has been a steady decrease since the 1940s, interrupted only by peaks before and after the two world wars. The shift began before intensive industrialisation and before the widespread introduction of pesticides and hormone-based medicines, leading the authors to conclude that a causal connection is less likely.[52] Similar analyses are not yet available for Germany.

**Assessment:**
Although a shift towards females in the birth ratio cannot automatically be termed a negative effect on human health, this parameter is a sensitive indicator for the hormonal environment in early pregnancy. The shift indicates changes in this environment. However, hypothesising an influence from endocrine disrupters does not explain the pre-industrial shift.

***2.4 Behavioural change and endocrine disrupters:***

A summary of the key discoveries as to the influence of PCB on behavioural parameters in new-born babies and children can be found in a recent article by Winneke[53]:

*"PCB can enter the placenta and therefore expose the human foetus to contaminations of the maternal fatty tissue. After birth, the baby is exposed to relatively high concentrations of PCB through maternal milk. As a potential hazard in development, these concentrations of PCB in lactate have received significant attention. After the ban on producing and using PCB, concentrations in human milk have fallen steadily since the mid-1980s, albeit more slowly than those of other organochlorine compounds. As for the range of biological effects – enzyme induction, immunotoxicity, reproductive toxicity and thyroid subfunction – experimental and epidemiological results imply that the compounds' neurotoxicity with respect to development plays a prominent role[54]. There are estimates that increases in environmental concentration can have a toxicological significance for the developing nervous system in the upper 10% of a typical distribution of the general public [55].*

*The significance of PCB pollution's toxicity to the developing human nervous system is principally justified by the results from the Michigan study[56,57,58], a cohort in North Carolina[59,60], the outcome of a toxic incident in Taiwan[61,62] and two cohorts in the Netherlands[63,64].Although all the studies describe negative effects on neurological or cognitive development as a result of early exposure to PCB, the findings are by no means consistent in many important aspects, particularly the range and persistence of observed deficits[65].*

*Because of these inconsistencies, a Europe-wide study co-ordinated by the MIU [Med. Institute for Environmental Hygiene at Düsseldorf University – ed.] and involving two groups from the Netherlands and one from Denmark, was begun with support from the EU. The project was funded from 1993 to 1999, and studied the neurological and cognitive/motor development of new-born infants of various ages, and some preliminary publications are now available[66,67,68,69]. The results may be summarised as follows: negative consequences from pre- or perinatal PCB pollution are not pronounced up to an age of 18 months, except in isolated cases [which still lie within normal bounds – ed.], while clearer links between poor motor and mental development and early exposure to PCB can be shown for ages between 30 and 42 months.*

*Alongside their neurotoxicity, PCB's potential for interaction with the endocrine system has recently been receiving more attention[70]. As well as effects on thyroid hormones, which are*

*under discussion as a possible cause of the developmental neurotoxicity, other forms of interaction with sexual hormones are clearly also especially significant. New animal experiments, conducted by Lilienthal and co-workers within a PUG-funded project (Project Environment and Health – ed.), were able to show significant and persistent antiandrogenic effects of PCB on both the endocrine system and the behaviour of rats[71]. Comparable results in humans are scarce, one of the few being the findings of the Taiwan study, where it could be shown in a matrix test with matched control children that boys exposed to PCB were considerably more seriously affected than girls. The authors take this result to be an indication of estrogenic or antiandrogenic effects from PCB during early development of the brain. Further (weak) evidence of PCB interaction with sexual steroids are the shorter penises of the exposed boys in the Taiwan cohorts, as well as the positive link, observed by Lanting[66], between maternal exposure to PCB and the quantities and fat content of their lactation."*

**Assessment:**
The link between PCB in maternal milk and children's' cognitive abilities can be considered proven in Germany as well. The findings are significant, but the deviations are within normal parameters. The role of PCB in obstructing transplacental transport of thyroid hormones is being debated, and animal experiments support the hypothesis. These findings require special attention to substances which affect the thyroid hormone system. Links to other xeno-estrogens are unknown.

***2.5 Final assessment of the results on harm to human health***

In Germany, the factors discussed above indicate that endocrinally active substances do affect the health and development of humans, although there is as yet no proof of causality. Research projects are attempting to collect quality *in vivo* data (including pharmakokinetics) on at least a few synthetic substances and selected phytoestrogens, to support an evaluation of the causality and the risks.

In principle, however, the changes discovered are consistent with the hypothesis that endocrine disrupters have played a part. Methodological problems, especially the long latency between exposure and effects and the complicated modes of exposure (present, past, *in utero*) make determining potential causality difficult. In addition, the effects of simultaneous exposure to estrogens and antiestrogens, for example, cannot be assessed at present.

Many of these unanswered questions reveal that the instruments for observing the environment and public health in Germany are inadequate.

In particular, there is no perinatal archive, which would make it possible to study the exposure of new-born babies retrospectively. Alongside environmental and health surveys of adults and children, there is no program to record data on umbilical blood or placenta samples or on human lactate, in order to study the exposure of new-born babies prospectively or

retrospectively. The Environmental Specimen Bank could be extended with such a perinatal archive.
The study by Rösch[22] has shown the importance of a nation-wide malformation register for new-born babies, with validated and detailed records of malformations. Monitoring the early phases of life makes it easier to discover the potential causal environmental exposure.

# 3. Contamination of environmental media and harm to ecosystems from endocrine disrupters

## *3.1 Harm to aquatic ecosystems*

The majority of information as to chemical disruption of the endocrine systems of wild organisms in Germany is from aquatic ecosystems. This is due to the fact that most research projects have concentrated on this medium. But there is no reason to suppose that there is no impact on terrestrial ecosystems.

**Invertebrates:**
The increased incidence of certain disruptions in the fertility and development of marine and limnetic molluscs, such as imposex and intersex formation in prosobranchia gastropods, or shell malformation and disruption of larval development in oysters, are considered the direct effects of aquatic pollution by organic tin compounds, especially tributyltin (TBT). TBT is a non-steroidal compound which is used in, for example, antifouling paint for ships. Direct correlations between the imposex and intersex stages and TBT pollution in those areas were found above all in the vicinity of harbours[72,73].

Using the areas of ship's hull coated with antifouling paint in the German merchant navy and a leaching rate of 1 mg/cm$^2$/d TBT as a basis, it was estimated that 45 tonnes of TBT are discharged into the North Sea per annum. Assuming even distribution, this would therefore produce in theory a total concentration of c. 0.24 ng/l. Measured concentrations of organic tin in German Bight were in fact 1.2 ng/l, in coastal waters 7.6, 6.0 and 2.6 ng/l and in the central North Sea between $\geq$ 1 and 0.5 - 0.7 ng/l[74].

The biological effect is expressed through androgen mechanisms preventing the formation of estrogen and inducing male characteristics in female snails. This effect, associated with a significant increase in endogenous testosterone content, can be shown at TBT concentrations of 5 ng/l (as Sn).[75] Depending on the level of toxicity, the animals' reproductive function may be impaired due to anatomical malformation or the animals may become completely sterile.

The periwinkle *Littorina littorea* and dogwinkle *Nucella lapillus* (North Sea), and the mud snail *Hydrobia ulva* (Baltic Sea) are being observed as indicator organisms. Imposex formation occurs in *Nucella lapillus* at concentrations as low as 1 - 3 ng TBT/l.[76]

The seriously threatened stocks of the common whelk *(Buccinum undatum)* in the North Sea[77], which occur predominantly in sediment in open seas, appear to be recovering slowly, as a consequence of the restriction in the use of organic tin compounds[78]. The lowest NOEC for this species is for reproductive functions, at 8.3 ng TBT/l (measured over 8 months, based on imposex formation)[79].

On the basis of the framework provided for quality standards in the proposed EU water framework guideline, the existing ecotoxicological data were evaluated and the target set for the protection of aquatic habitats from harm from tributyltin compounds at 0.1 ng/l, in terms of tributyltin cations[80].

Samples of *Nucella lapillus* with pronounced imposex collected in 1993-1995 from Norwegian coastal waters contained 48-1096 ng Sn/g TS. Here too, there was a direct correlation between TB content in the animals and the extent of imposex formation. The NOEC at which no malformation could be found was below the discernible limit of 7 ng Sn/g dw, and was determined graphically as 4 ng Sn/g dw[81].

Over 70 marine species are affected by this phenomenon around the world[82].

**Summary:**
Limnic and marine mollusc species in Germany are exposed to triorganic tin concentrations which give reasons to fear significant harm. Stocks of these species have fallen drastically in recent decades. Contamination by TBT must therefore be assumed to be the cause.

**Vertebrates:**

Tests on fish from German inland and coastal waters show increased incidence of high concentrations of vitellogenin (preproduct of vitellus) in their blood, regardless of their gender or the time they were caught. Vitellogenin is an indicator for contamination by estrogens and substances with estrogenic effects.[83]

Organisms which are induced to synthesise vitellogenin by external estrogenic stimuli also exhibit other more or less pronounced negative effects, including, among others, a shift in steroid metabolism, atrophy of the liver, delayed testicle growth with frequent occurrence of ova in male testicles (ovotestis) in juvenile and adult males, as well as disruption of gamete production associated with reduced reproductive success[84,85,86]. Since male organisms lack the

target organs for vitellogenin, it is retained in the blood, which can cause damage to the kidneys and calcium deficiency. In addition, this additional abnormal biosynthesis, especially where the fish is exposed to a high level of estrogenic stimulants, depletes the energy and disrupts the hormonal equilibrium in the animal's body [87,88,89,90]. In females, excessive levels of estrogens and estrogenic substances cause prematurity and abnormal development of ovaries and ova, reducing the likelihood of eggs hatching successfully[91].

Another indicator for excessive levels of estrogen or estrogenic substances under discussion is a shift in the gender ratio towards females in fish populations, as well as the increased occurrence of ova in male testicles (ovotestis).

Although the phenomenon of ova forming in fish testicles can frequently be observed both in fish farms and in the wild, carefully designed studies have shown that increasing concentrations of estrogens or estrogenic pollutants can increase the number of hermaphrodites, including fully developed female sexual characteristics[92]. This is also exploited in fish farms, in order to produce faster growing female fish.

Long term exposure of adult male trout and carp to gradually increasing amounts of domestic waste water from a Berlin treatment plant demonstrated a quantitative connection to induced synthesis of vitellogenin in the blood. The spawn from this test series was exposed to the same pollution as the parent fish for a further 12 months after the larvae hatched. The sexual characteristics of the offspring shifted increasingly towards almost complete feminisation where the waste water concentration was at 40 %[93,94]. This level of concentration is certainly possible in Berlin surface water during the summer months.

Studies of fish stocks in Berlin's water bodies conducted by the fisheries agency between 1985 and 1995 have found shifts in gender ratios among certain species (zander, roach, asp), while other species seemed scarcely affected. The catches were made during stock management, aimed primarily at screening out fast-growing and less profitable species. It is therefore possible that the generally larger females were caught in the study . On the other hand, analysis of the catch data in terms of water pollution, using zander caught in 1998 as an example, showed a significantly larger proportion of females in more heavily polluted regions.[95]

However, a study of the gender ratios and gonad structures of roach *(Rutilus rutilus)* and common perch *(Perca fluviatilis)* from the Spree and the Havel, made in the same year, showed no abnormal results[95].

In North Rhine-Westphalia, brace from the Rhine were compared with a related species from the Wahnbach reservoir. A histology of the testicles found ovotestis in only three out of 59 fish from the Lower Rhine, and none at all in the brace from the reservoir. The gender ratio of both catches was balanced. On the other hand, the vitellogenin content of blood plasma was four times as high in male brace from the Rhine as in those from the Wahnbach reservoir (980 and 225 µg/l)[96]. The levels of vitellogenin in the reservoir fish were also raised, which implies some contamination through estrogen-like chemicals.

Vitellogenin tests in male brace from polluted sections of the Elbe showed only slightly higher levels up to 200 µg/l, i.e. comparable levels to those found in the Wahnbach reservoir. Of 97 male fish examined, only 5 had oozytes[97,98].

These results permit us to conclude that increased vitellogenin levels in the blood of fish or shifts in the gender ratio of populations can be used as biomarkers for estrogenic contamination, although a combination of vitellogenin levels and other parameters (e.g. histological change, induced mixed function oxidases or concentrations of steroids) is recommended for evaluating specific pollution situations[98,99].

**Summary:**
The data show that there is widespread pollution by estrogenic substances in Germany's surface water, which lead to negative change in fish. Induced vitellogenin synthesis can be used as a biomarker for these adverse effects.

### *3.2 Incidence of endocrine disrupters in water bodies*

In the publication"Substanzen mit endokriner Wirkung in Oberflächengewässern", UBA Texte 46/97 knowledge in the literature on over 200 suspected endocrinally active substances in the environment was collated. Their endocrinal effects were evaluated, as well as their actual importance for water quality, using measurements from a survey of German Länder, databases at the UBA and from local authorities, and information about the production and environmental behaviour of the chemicals. However, the importance of the substances for water quality proved difficult to assess, as for most substances there are no measurements, and the relative potency of the substances is unknown.

The following chemicals appear to have a special relevance:

1 Alkylphenolethoxylates and their metabolites and decomposition products
Nonylphenol and octylphenol, as well as the decomposition products of nonylphenolethoxylates, NP1EO and NP2EO induce vitellogenin synthesis in male

and female fish. The lowest observed effect concentration (LOEC) for octylphenol is 5 µg/l, for nonylphenol 20 µg/l, for NP1EO and NP2EO approximately 30 µg/l. Analytic studies have shown that nonylphenol concentration in unpolluted stretches of river are between $\leq$ 0.01 and 0.1 µg/l. Downstream from sewage treatment plants, and depending upon population density and industrial structure, concentrations between 0.7 and 16.5 µg/l nonylphenol are found. The sediment of these stretches contains concentrations of 1 to 156 mg/kg. Concentrations of octylphenol and NP2EO, even in water with a heavy load of sewage, are generally an order of magnitude below their LOEC, although peak concentrations of NP1EO have been measured in the range of its effect concentration. The tests were random samples, and the results cannot therefore be used to determine a general trend in pollution levels. Tests between 1988 and 1991 in Bavaria nonetheless showed an average 50 % decrease in water pollution[100].

2 The effects of tributyltin (TBT), an androgenic substance, have been observed on water snails in field studies. Laboratory tests have shown that the development of male sexual organs in female snails (pseudohermaphroditism or imposex) is triggered by a rise in the testosterone titre resulting from disruption of the hormone synthesis by TBT (LOEC 0.005 µg/l TBT Sn for marine snails, 0.08 µg/l TBT Sn for limnic snails). TBT is used predominantly as a biocide in antifouling paints for ships. Its use has been banned, but only for boats under 25 m, since 1990. Elevated concentrations of TBT are still being found in sediment and suspended matter in German rivers. Between 1987 and 1990 an UBA research project found maximum concentrations of c. 1 µg/l tributyltin (0.41 µg/l TBT Sn) in various marinas on the Bodensee, in Berlin, Hamburg and Kiel. Median concentrations in fresh water were 0.025 µg/l (0.010 µg/l TBT Sn), in the Baltic c. 0.150 µg/l (0.06 µg/l TBT Sn) and in the North Sea c. 0.080 µg/l (0.033 µg/l TBT Sn)[101]. A number of tests conducted on suspended matter by the *Länder* and local authorities showed high concentrations within the range of effect concentrations, the Elbe and its tributary the Mulde proving most heavily polluted. Also of note were the high concentrations of other butyltin compounds, caused by industrial discharges. The target for protection of aquatic habitats is 0.1 TBT Sn ng/l in water and 2 µg/kg TBT Sn in suspended matter. Every measuring station on the Elbe and Mulde where the analysis was sufficiently sensitive showed concentrations in excess of these targets.

Samples of brace (*Abramis brama*) and zebra mussels (*Dreissenia polymorpha*) collected between 1992 and 1998 for the Environmental Specimen Bank from the Rhine, Elbe, Saar, Mulde, Saale and the Belauer Lake (Bornhöveder Lake District)

were tested for organic tin compounds in a research and development project (Tab. 1 and 2)[102]. Increased levels of tetrabutyltin (TTBT), tributyltin (TBT), dibutyltin (DBT), monobutyltin (MBT) and triphenyltin (TPhT) were measured. Levels of monooctyltin (MOT), diooctyltin (DOT) and tricyclohexyltin (TCxT) were generally below the analytical detection limit. In the Elbe and the Rhine, the concentrations increase in downstream samples. The highest TBT concentration was found in samples from the sample point Blankenese/Elbe. Samples from the Saar were relatively uncontaminated, relatively high concentrations of TPhT were found in muscle tissue from brace samples from the Belauer Lake.

**Tab. 1:** Organic tin compounds in zebra mussels from the Elbe, Rhine and Saar (Environmental Specimen Bank, in µg Sn/kg round weight)

| **River** | **MBT** | **DBT** | **TBT** | **TTBT** | **TPhT** | **Σ Sn** |
|---|---|---|---|---|---|---|
| **Elbe** (1996) (1 PNF) | 8 | 4 | 385 | 4 | 5 | 408 |
| **Rhine** (1996) (4 PNF) | <1-2 | <2 | 2-6 | <1 | <2-4 | 4-9 |
| **Saar** (1995)(2 PNF) | <1/2 | <2/<2 | 3/6 | <1/<1 | <2/<2 | 5/6 |

**Tab. 2:** Organic tin compounds in brace muscle tissue from the Elbe, Saale, Mulde, Belauer Lake, Rhine and Saar (Environmental Specimen Bank, in µg Sn/kg round weight)

| **River** | **MBT** | **DBT** | **TBT** | **TTBT** | **TPhT** | **Σ Sn** |
|---|---|---|---|---|---|---|
| **Elbe** (1998) (3 PNF) | <1 | 2-11 | 12-168 | 7-13 | <2-26 | 14-217 |
| **Saale** (1998) (1 PNF) | <1 | <2 | 18 | <1 | <2 | 18 |
| **Mulde** (1998) (1 PNF) | <1 | 4 | 32 | 8 | 6 | 50 |
| **Belauer Lake** (1997) | <1 | <2 | 1 | <1 | 9 | 10 |
| **Rhine** (1998) (4 PNF) | <1 | <2 | 5-10 | <1 | <2-18 | 5-32 |
| **Saar** (1995)(2 PNF) | <1 | <1 | 6/7 | <1 | <2 | 6/7 |

Between 1993 and 1998, an average downwards trend was found in TBT concentrations in brace from inland river sample points. This does not apply to the mouth of the Elbe (Blankenese), where they remain consistently high, presumably due to the influence of the docks and merchant shipping. In contrast, a rise in TPhT concentrations was found everywhere but at Blankenese, indicating greater use of TPhT as a crop protection agent.

Organic tin compounds were also measured in water and sediment from selected rivers[103]. High concentrations were found in the Elbe, Mulde and Rhine. The sedimentary concentrations in the most notable river sample points are shown in Table 3.. Concentrations of TTBT appear to be falling between 1994 and 1996, while concentrations of TBT and TPhT remain almost unchanged. The data for water samples are sporadic, the range of TBT concentrations measured at Ems-Herbrum in 1996 was between < 0.002 and 0.002 µg/l. This was a cause for concern, as the target maximum for protecting aquatic habitats (2 ng/l) is exceeded by a factor of 20.

**Tab. 3:** Organic tin compounds in sediment from the Elbe, Rhine and Saale (in µg/kg dry weight)

| River | | TBT | TTBT | TPhT |
|---|---|---|---|---|
| **Elbe** | (1996) Schnackenburg | <1-80 | <1-140 | <1 |
| **Saale** | (1995)Groß Rosenburg | 4-53 | <1-57 | <1 |
| **Mulde** | (1996) Dessau | 73-427 | 240-2420 | <1 |
| **Rhine** | (1996)Kleve-Bimmen | 12-85 | <2 | <2-7,3 |

Samples from the Environmental Specimen Bank of bladderwrack (*Fucus vesiculosus*), marine mussels (*Mytilus edulis*), eelpout (*Zoarces viviparus*) and herring gull (*Larus argentatus*) eggs, collected from areas along the North Sea and Baltic coasts, were tested for organic tin compounds in a research and development project[102] (Tab. 3 and 4). Elevated concentrations of tributyltin (TBT), dibutyltin (DBT), monobutyltin (MBT), diphenyltin (DPhT), and triphenyltin (TPhT) were found. Levels of monooctyltin (MOT), diooctyltin (DOT) and tricyclohexyltin (TCxT) were generally below the detection limit of the study. Tables 4 and 5 show only the levels in marine mussels and eelpout muscle tissue, as herring gull eggs and bladderwrack contain relatively low levels of organic tin compounds.

**Tab. 4:** Organic tin compounds in marine mussels from the North Sea (Eckwarderhörne) and the Baltic Sea (Darßer Ort) (Environmental Specimen bank, in µg Sn/kg round weight)

| | MBT | DBT | TBT | DPhT | TPhT | Σ Sn |
|---|---|---|---|---|---|---|
| **North Sea (1996)** | 2 | <2 | 8 | <1 | 3 | 14 |
| **Baltic Sea (1996)** | 3 | <2 | 7 | <1 | <2 | 10 |

**Tab. 5:** Organic tin compounds in eelpout muscle tissue from the North Sea (Jadebusen) and the Baltic Sea (Darßer Ort) (Environmental Specimen bank, in µg Sn/kg round weight)

| | MBT | DBT | TBT | DPhT | TPhT | Σ Sn |
|---|---|---|---|---|---|---|
| **North Sea (1998)** | <1 | <2 | 4 | <1 | 2 | 6 |
| **Baltic Sea (1998)** | <1 | <2 | 18 | <1 | <2 | 18 |

Over the years, TBT concentrations have remained more or less constant in marine mussels (1985-96) and eelpout (1994-98). The source of TBT is presumably merchant shipping. In contrast, TPhT concentrations have fallen by at least a half.

There is evidence for alarmingly high concentrations of organic tin compounds in marine mammals. High levels of TBT have been found in the blubber and livers of dolphins and whales (e.g. the finless porpoise *Neophocaena phocaenoides*: 770 µg TBT/kg round weight).[104] A fall of concentrations from the coast to the open seas has been noted. Apart from the levels of TBT found in blubber and liver, the substance also appears to accumulate in the animals' central nervous system (brain).

High concentrations of organic tin compounds are found particularly in the vicinity of dischargers such as harbours. After the ban on organic tin compounds in antifouling paint, a clear fall in TBT levels was observed (presumably because leisure boats were the primary source). However, although levels in areas far from emittants are significantly lower, organic tin compounds can be found in organisms in the remotest high sea and deep sea areas.[105].

- Bisphenol A
  Bisphenol A is a chemical with estrogenic effect. Its feminising effect has also been shown in male trout. Significant quantities (1995: 210,000 t) are produced in Germany, primarily for use in plastics manufacture. At the time of the study there were no data on its incidence in German waters, and specific tests conducted in various *Länder* showed pollution of surface waters of < 1 µg/l (frequently < 10 ng/l, in the Elbe and the Saale c. 100 ng/l).

- Phthalic acid esters
  Because of their widespread use as plasticizers in PVC, phthalates are ubiquitous. Extensive data on their incidence in the environment have been published by the UBA in the report "Action Areas and Criteria for a Precautionary, Sustainable Substance Policy Using the Example of PVC" (in English: February 2001).

- Gamma-HCH
  A follow-up study showed that vitellogenin synthesis in juvenile fish was stimulated by Gamma-HCH at concentrations in excess of 0.1 mg/l or 0.18 mg/l[106]. The maximum concentration measured between 1993 and 1996 in the LAWA measuring network was 0.6 µg/l[107]. The target set by International Commission for the Protection of the Rhine (ICPR) for Gamma-HCH is 0.1 µg/l, according to current knowledge, the substance's toxic effects are greater than its endocrine effects[108].

The above discussion concentrates on a number of important synthetic substances. To assess the total contamination of waters with endocrine disrupters, naturally excreted hormones (form humans and animals) and synthetic hormones must also be considered (Chapter 4.3).

# 4. Endocrine disrupters in the environment and possibilities for identifying them

## *4.1 Procedures for testing endocrine effects*

Much work is still in progress within the OECD on validating and standardising testing procedures to determine the endocrine effects of chemicals. The present information on the endocrine effects of chemicals is gathered from:

- *in vitro* tests of organs, cells or subcellular structures,
- *in vivo* tests using standard experimental methods,
- *in vivo* tests using standard procedures on e.g. reproduction, whose goals are not the investigation of hormonal mechanisms, but which can provide indirect hints as to these effects.

The following basic requirements are essential for procedures to investigate the endocrine activity of chemicals:

- suitability for identifying effects on the endocrine system,
- meaningfulness for intact organisms,
- relevance of the results for other organisms,
- reproducibility of results.

*In vitro* tests examine the effects of chemicals on cells, subcellular structures or certain organs or tissue types. Such relatively simple procedures are useful in determining effects (e.g. binding to hormone receptors, egg maturation or induced synthesis of certain endocrinally regulated proteins or RNA). However, extrapolating the results to endocrine effects on the organism as a whole is not possible, as the procedure ignores resorption, distribution and the possible metabolisation or excretion of the substance.

There is therefore a consensus in Europe that no final assessments of chemicals should be made on the basis of *in vitro* tests when drafting regulations.

The utility of *in vitro* tests in screening is also disputed by experts, as expressed in the OECD's "Draft Detailed Review Paper: Appraisal of Test Methods for Sex-Hormone Disrupting Chemicals", for example. In general, *in vitro* tests could be suitable for priority-setting, i.e. identifying substances which are then subjected to more detailed examination in *in vivo* assays. Apart from producing reproducible results, the tests should also meet the following requirements:

- Few false positive results, i.e. a positive result should be associated as strongly as possible with an endocrinal mechanism.

- Few false negative results, i.e. a high percentage of suspect substances should be identified with sufficient sensitivity. As there are several mechanisms by which chemicals can affect the endocrine system, this requirement cannot be met by a single test, but only by a battery of tests.

Further toxokinetic discoveries (absorption, excretion, distribution, metabolism) can then justify further tests. For priority-setting, therefore, the UBA considers further development and validation of *in vitro* assays for substance testing to be sensible.

**Excursion:** Combining *in vitro* tests with chemical analysis:

A method developed by B. Hock (TU Munich), "effect analysis", could help in identifying high-priority substances. Suspect substances in natural water samples are allowed to bind to fixed estrogen receptors, isolated, analysed and quanitified.[109] The method is suitable for recognising potentially estrogenic or antiestrogenic substances which are present in the environment, and which can then be further tested and evaluated.

Numerous already standardised *in vivo* testing procedures (e. g. OECD, US EPA, FIFRA, IOBC and EPPO testing guidelines) are not directly conceived for endocrine effects, but may provide indirect evidence::

Mammals:
- Subacute toxicity (28 days exposure)
- Subchronic toxicity (90 days exposure)
- Chronic exposure (18 or 24 months exposure) usually of 2 species
- Multigeneration study
- Teratogenicity test on 2 species

Birds:
- Reproduction studies of quails

Fish:
- Early life-stage test (e.g. *Danio rerio)*
- Full life-cycle test (e.g. *Danio rerio*)

Aquatic invertebrates:
- Daphnia reproduction test

Sediment organism tests on chironomides (larval development)

Terrestrial invertebrates:

*Aleochara bilineata* (rove beetle): reproduction

*Aphidius rhopalosiphi* (parasitic wasp): reproduction

*Chrysoperla carnea* (green lacewing): fertility

*Coccinella septempunctata* (7-spotted ladybird): fertility

*Folsomia candida* (springtail): reproduction

*Poecilus cupreus* (ground beetle): larval development

*Syrphus corollae* (hover fly): fertility

*Trichogramma cacoeciae* (egg parasitoid): parasitic performance and fertility

*Typhlodromus pyri* (predatory mite): fecundity and fertility

*Eisenia foetida* (compost worm): reproduction

However, the most valuable tests are *in vivo* tests that deliberately set endpoints connected with endocrine activity, to investigate phases of life which are expected to be especially sensitive. Numerous substances now considered as endocrinally active have been identified in such tests, which are valid, if not yet standardised. These include reproduction studies in Collembola and lacewing, levels of vitellogenin synthesis in fish, metamorphosis in amphibians, as well as numerous studies on mammals. Some particularly suitable tests have been selected for standardisation by the EDTA (Endocrine Disrupter Testing and Assessment) working group, a task force set up as part of the OECD test guidelines programme.

A Validation Management Group (VMG) was set up in 1998 to validate new and redesigned methods for testing endocrine effects on mammals. Work is currently focused on validating two short-term tests for identifying estrogenic and androgenic effects (Uterotrophic and Hershberger assay), as well as an extended 28-day test on oral toxicity to rats under repeated doses (enhanced TG 407). A date for the conclusion and evaluation of the extensive tests cannot yet be set.

For ecotoxicity testing methods, the EDTA Task force is focusing on endocrine effects on fish, although progress in test guidelines for bird reproduction are in prospect. To co-ordinate further development and validation, a “VMG-eco”, similar to the “VMG-mammalian”, has recently been decided upon. The second OECD meeting of fish experts recommended developing and validating a short-term test for young and adult fish, a test in early life-stage (based on the OECD 210 test), a reproduction test and a full life-cycle test. Germany is playing an active role here. Work on bird reproduction is waiting upon the conclusion of a comparison between the sensitivity of various quail species. In the medium term, the need for and suitability of amphibian tests (e.g. African clawed frog test) will be examined.

With a research project, on developing a biological test on *Marisa cornuarietis* (Gastropoda: Prosobranchia) to determine endocrine disrupters in the environment, the UBA has given impetus to the development of such tests. Results to date point to the potential high sensitivity of such testing systems, and not only to triorganic tin compounds. It should be noted that the validation requirements have not yet been met for these results, and the data cannot therefore be used for regulatory purposes as yet.

### *4.2 Endocrine disrupters in the environment*

Gülden et al. have published a list of substances in surface waters suspected of being endocrine disruptors.[2] This list of over 200 substances contains many for which a definitive judgement is impossible, due to a lack of valid *in vivo* studies.

It should be emphasised that even substances definitively classed as endocrine disrupters are more or less a random selection, as systematic, large-scale testing programmes are lacking (also a consequence of there being no standardised methods). Furthermore, the endocrine disrupters do not possess a limited set of clearly describable structural characteristics, making a prognosis as to the total number of disrupters impossible at this time

In June 2000, BKH Consulting Engineers Delft and TNO Nutrition and Food Research Zeist, in the Netherlands, published a report commissioned by the EU Commission (DG ENV): "Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption". These results were presented by the EU Commission at the Joint Meeting of the Competent Authorities (DOC/ENV/D 720257/00 NOTIF/23/2000) on 31/5/2000. A four-tier selection procedure on 564 substances identified 60 high-priority substances, which had been proved endocrinally active in at least one *in vivo* study, are considered either persistent or substances with a high production volume, and where exposure of humans or the environment can be presumed. On 8$^{th}$ and 9$^{th}$ November 2000, an expert meeting considered these results, and asked the Commission to rapidly develop a schedule for further steps, and especially to complete the data on substances which are as yet only classified as potential endocrine disrupters, in order to classify their priority as fast as possible. The substances are listed in the following Table 6.

**Table 6: High priority endocrine disrupters**

| No | Substance name | CAS No |
|---|---|---|
| 1 | Chlordane | 12789-03-6 |
| 2 | Chlordane (cis- and trans-) | 57-74-9 |
| 3 | Kepone (Chlordecone) | 143-50-0 |
| 4 | Mirex | 2385-85-5 |
| 5 | Toxaphene = Camphechlor | 8001-35-2 |
| 6 | DDT (technical) = clofenotane | 50-29-3 |
| 7 | p,p'-DDT = clofenotane | No CAS 008 |
| 8 | Tetrachloro DDT = 1,1,1,2-Tetrachloro-2,2-bis(4-chlorophenyl)ethane | 3563-45-9 |
| 9 | Vinclozoline | 50471-44-8 |
| 10 | Maneb | 12427-38-2 |
| 11 | Metam Natrium | 137-42-8 |
| 12 | Thiram | 137-26-8 |
| 13 | Zineb | 12122-67-7 |
| 14 | Gamma-HCH (Lindane) | 58-89-9 |
| 15 | Linuron (Lorox) | 330-55-2 |
| 16 | Atrazine | 1912-24-9 |
| 17 | Acetochlor | 34256-82-1 |
| 18 | Alachlor | 15972-60-8 |
| 19 | Styrene | 100-42-5 |
| 20 | Hexachlorobenzene (HCB) | 118-74-1 |
| 21 | Butylbenzylphthalate (BBP) | 85-68-7 |
| 22 | Di-(2-ethylhexyl)phthalate (DEHP) | 117-81-7 |
| 23 | Di-n-butylphthalate (DBP) | 84-74-2 |
| 24 | 2,2-Bis(4-hydroxyphenyl)propan = 4,4'-isopropylidenediphenol = Bisphenol A | 80-05-7 |
| 25 | PCB | 1336-36-3 |
| 26 | PCB 153 (2,2',4,4',5,5'-Hexachlorobiphenyl) | 35065-27-1 |
| 27 | PCB 169 (3,3',4,4',5,5'-Hexychlorobiphenyl) | 32774-16-6 |
| 28 | PCB 47 (2,2',4,4'-Tetrachlorobiphenyl) | 2437-79-8 |
| 29 | PCB 77 (3,3',4,4'-Tetrachlorobiphenyl) | 32598-13-3 |
| 30 | PCB Aroclor 1242 | 53469-21-9 |
| 31 | PCB Aroclor 1248 | 12672-29-6 |
| 32 | PCB Aroclor 1254 | 11097-69-1 |

| | | |
|---|---|---|
| 33 | PCB Aroclor 1260 (Clophen A60) | 11096-82-5 |
| 34 | PBBS = Brominated Flame retardants = PBB (mixed group of 209 Congeners) | 59536-65-1 |
| 35 | 1,2,3,7,8-Pentachlorodibenzodioxin | 40321-76-4 |
| 36 | 2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) | 1746-01-6 |
| 37 | 2,3,7,8-TCDF | 51207-31-9 |
| 38 | Tributyltin compounds | No CAS 050 |
| 39 | Tributyltin hydride | 688-73-3 |
| 40 | Tributyltin oxide = bis(tributyltin) oxide | 56-35-9 |
| 41 | 2-propenoic acid, 2-methyl-, methyl ester = Stannane, tributylmethacrylate | 26354-18-7 |
| 42 | Methoxyethylacrylate tributyltin, copolymer | No CAS 100 |
| 43 | Phenol, 2-[[(tributylstannyl)oxy]carbonyl]- | 4342-30-7 |
| 44 | Stannane, (benzoyloxy)tributyl- | 4342-36-3 |
| 45 | Stannane, [1,2-phenylenebis(carbonyloxy) | 4782-29-0 |
| 46 | Tributyltin naphthalate | 36631-23-9 |
| 47 | Stannane, tributyl-, mono(naphthenoyloxy) | 85409-17-2 |
| 48 | Stannane, tributyl[(1-oxo-9,12-octadecadienyl)oxy]-, (Z,Z)-) | 24124-25-2 |
| 49 | Stannane, tributyl[(1-oxo-9-octadecenyl)oxy]-, (Z)- | 3090-35-5 |
| 59 | Stannane, tributyl[[[1,2,3,4,4a,4b,5,6,10,10a-decahydro-1,4°-dimethyl-7-(1-methylethyl)-1-phenanthrenyl]carbonyl]oxy]-, [1R-(1.alpha., 4a.beta., 4b.alpha.,10°.alpha.)]- | 26239-64-5 |
| 51 | Stannane, tributylfluoro- | 1983-10-4 |
| 52 | Tributyl[(2-methyl-1-oxo-2-propenyl)oxy]stannane | 2155-70-6 |
| 53 | Tributyltincarboxylate | No CAS 099 |
| 54 | Tributyltinnaphthalate | 26636-32-8 |
| 55 | Tributyltinpolyethoxylate | No CAS 101 |
| 56 | Tri-n-propyltin (TPrT) | 2279-76-7 |
| 57 | Triphenyltin | No CAS 051 |
| 58 | Fentin acetate = triphenyltin acetate | 900-95-8 |
| 59 | 3,4-Dichloroaniline | 95-76-1 |
| 60 | Resorcinol | 108-46-3 |

While the significance of these substances as endocrine disrupters - i.e. their inclusion in Category I - is more or less clear, the exclusion of six Category I substances because of presumed low exposure levels has no reasonable justification. Listed in Table 7, these substances should continue to be considered high-priority.

| **Table 7: Category I substances which, because of presumed low exposure, are not considered priorities** | | |
|---|---|---|
| 1. | Amitrol | 61-82-5 |
| 2. | 4-tert. Octylphenol | 140-66-9 |
| 3. | 4-Nonylphenol | 25154-52-3 |
| 4. | Nitrofen | 1836-75-5 |
| 5. | Tetrabutyltin | 1461-25-2 |
| 6. | 4-Nitrotoluene | 99-99-0 |

Also, the following substances should be considered as essentially high-priority (category I):

| | |
|---|---|
| Alkylphenolethoxylates (APEO) | decompose to Nonyl-/Octylphenol |
| Diuron | decomposes to 3,4-Dichloroaniline |
| Phenanthrene, Chrysene, Benzanthracene, Dibenz[a,h]anthracene | there is a positive *in-vivo* test (Allen-Doisy-Test: estrogenicity in rodents) for these PAH. [110] |

Further discussion of these 70 substances should first examine the extent to which there is a need for any action at EU level. This is not the case for substances which are either already banned (e.g. Chlorodane, Mirex, DDT and its metabolites, PCB) or whose emission as unintentional by-products is already widely restricted (e.g. polychlorinated dioxins and furanes, or polycyclic aromatic hydrocarbons, PAH). Table 8 lists the remaining substances or substance groups and their main areas of application.

| **Table 8: Regulatory status of remaining Category I substances** | | | |
|---|---|---|---|
| **No** | **Substance name** | **Key area(s) of application** | **Regulatory status** |
| 1 | Vinclozoline | crop protection agent | EC-Reg. 3600/92; decision open. Permitted in Germany until 2002 |
| 2 | Maneb | crop protection agent | EC-Reg. 3600/92; Monograph to be finished. Permitted in Germany until 2008 |
| 3 | Metam-Sodium | crop protection agent | Not permitted in Germany (App 3 of the Crop Protection Agent Use Ordinance) |

| | | | |
|---|---|---|---|
| 4 | Thiram | crop protection agent | EC-Reg. 3600/92; decision open. Permitted in Germany until 2007 (as stripper) |
| 5 | Zineb | crop protection agent | EC-Reg. 3600/92; Monograph to be finished. Not permitted in Germany |
| 6 | Gamma- HCH (Lindane) | Crop protection and pest control agent | *crop protection agent:* EC-Reg. 3600/92; Proposed decision: No inclusion in App. 1; Not permitted in Germany (App 3 of the Crop Protection Agent Use Ordinance); *pest control agent:* permitted under § 10 c BSeuchG |
| 7 | Linuron (Lorox) | crop protection agent | EC-Reg. 3600/92; decision open. Not permitted in Germany |
| 8 | Atrazine | crop protection agent | EC-Reg. 3600/92; decision open. Not permitted in Germany (App 1 of the Crop Protection Agent Use Ordinance) |
| 9 | Acetochlorine | crop protection agent | Not permitted in Germany |
| 10 | Alachlorine | crop protection agent | EC-Reg. 3600/92; decision open. Not permitted in Germany |
| 11 | Styrene | industrial chemical (polymer pre-product) | 1st priority list under EU-Existing Substance Regulation 793/93/EC |
| 12 | Butylbenzylphthalate (BBP) | industrial chemical (plasticizer) | 3rd priority list under EU-Existing Substance Regulation 793/93/EC |
| 13 | Di-(2-ethylhexyl)phthalate (DEHP) | industrial chemical (plasticizer) | 2nd priority list under EU-Existing Substance Regulation 793/93/EC |
| 14 | Di-n-butylphthalate (DBP) | industrial chemical (plasticizer) | 1st priority list under EU-Existing Substance Regulation 793/93/EC |
| 15 | 2,2-Bis(4-hydroxyphenyl)propan = 4,4'-isopropylidenediphenol = Bisphenol A | industrial chemical (oxidation inhibitor, plastics additive) | 3rd priority list under EU-Existing Substance Regulation 793/93/EC |
| 16 | PBBS = Brominated Flame retardants = PBB (mixed group of 209 Congeners) | industrial chemical (flame retardant) | Polybrominted biphenyls no longer produced in Europe. a) |
| 17 | Tributyltin compounds | biocide (antifouling) | Banned for boats < 25 m long under Chemicals Prohibition Ordinance; comprehensice domestic ban under discussion |
| 18 | Tributyltin hydride | biocide (antifouling) | see above |
| 19 | Tributyltin oxide = bis(tributyltin) oxide | biocide (antifouling) | see above |
| 20 | 2-propenoic acid, 2-methyl-, methyl ester = Stannane, tributylmethacrylate | biocide (antifouling) | see above |

| | | | |
|---|---|---|---|
| 21 | Methoxyethylacrylate tributyltin, copolymer | biocide (antifouling) | see above |
| 22 | Phenol, 2-[[(tributylstannyl)oxy]carbonyl]- | biocide (antifouling) | see above |
| 23 | Stannane, (benzoyloxy)tributyl- | biocide (antifouling) | see above |
| 24 | Stannane, [1,2-phenylenebis(carbonyloxy) | biocide (antifouling) | see above |
| 25 | Tributyltin naphthalate | biocide (antifouling) | see above |
| 26 | Stannane, tributyl-, mono(naphthenoyloxy) | biocide (antifouling) | see above |
| 27 | Stannane, tributyl[(1-oxo-9,12-octadecadienyl)oxy]-, (Z,Z)-) | biocide (antifouling) | see above |
| 28 | Stannane, tributyl[(1-oxo-9-octadecenyl)oxy]-, (Z)- | biocide (antifouling) | see above |
| 29 | Stannane, tributyl[[[1,2,3,4,4a,4b,5,6,10,10a-decahydro-1,4°-dimethyl-7-(1-methylethyl)-1-phenanthrenyl]carbonyl]oxy]-, [1R-(1.alpha., 4a.beta., 4b.alpha.,10°.alpha.)]- | biocide (antifouling) | see above |
| 30 | Stannane, tributylfluoro- | biocide (antifouling) | see above |
| 31 | Tributyl[(2-methyl-1-oxo-2-propenyl)oxy]stannane | biocide (antifouling) | see above |
| 32 | Tributyltincarboxylate | biocide (antifouling) | see above |
| 33 | Tributyltinnaphthalate | biocide (antifouling) | see above |
| 34 | Tributyltinpolyethoxylate | biocide (antifouling) | see above |
| 35 | Tri-n-propyltin (TPrT) | Unclear | |
| 36 | Triphenyltin | crop protection agent, earlier: biocide (antifouling) | EC-Reg. 3600/92; decision open. Permitted in Germany until 2003 |
| 37 | Fentin acetate = triphenyltin acetate | crop protection agent | EC-Reg. 3600/92; decision open. Not permitted in Germany |
| 38 | 3,4-Dichloroaniline | Decomposition product of several PSM agents, industrial chemical (intermediate) | 1st priority list under EU-Existing Substance Regulation 793/93/EC (risk assessment skipped) |
| 39 | Resorcinol | industrial chemical | |
| 40 | Amitrol | crop protection agent | |
| 41 | 4-tert. Octylphenol | industrial chemical, pre- and decomposition product of emulsifiers and detergents, above all | |
| 42 | 4-Nonylphenol | industrial chemical, lubricant, pre- and decomposition product of emulsifiers and detergents, above all | 1st priority list under EU-Existing Substance Regulation 793/93/EC |
| 43 | Nitrofen | crop protection agent | Not permitted in Germany (App 1 of the Crop Protection Agent Use Ordinance) |
| 44 | Tetrabutylzinn | industrial chemical (intermediate for Tri-, Di- and Monobutyltin comp.) | |
| 45 | 4-Nitrotoluene | industrial chemical (intermediate) | |
| 46 | Diuron | crop protection agent | Permitted in Germany until 2008 (App 1 of the Crop Protection Agent Use Ordinance) |

| | | | |
|---|---|---|---|
| 47 | Alkylphenolethoxylate | industrial chemical (emulsifier, detergent) | Nonylphenolethoxylate covered by risk assessment of 4-Nonylphenol. |

a) The most important examples of structurally similar polybrominated diphenyl ether (PBDE), Penta-, Octa- and Decabromodiphenyl ether, are currently given priority in the European Existing Substances Programme under the EU Existing Substances Regulation 793/93/EC.

Where closer examination of these substances reveals significant exposure, they should be assessed rapidly, and regulated where necessary. The last column of Table 8 shows whether a risk assessment is already provided for in Directive 91/414/EWG or Regulation 793/93/EG. The assessments should include results about endocrinal activity.

However, the endocrine effect is not necessarily the decisive factor in the assessment. Some of the estimated (or calculated) exposure concentrations are far below the corresponding LOEC, and other toxic or ecotoxic effects may be more significant. Under the current guidelines, a substance's endocrine effects then have no influence on the result of the assessment, nor therefore on any necessary reduction measures. 4-octylphenol, resorcinol, tri-n-propyltin (compounds) and nitrotoluene are not currently on any priority assessment list. It is uncertain whether tripropyltin compounds (which are very similar to tributyltin) are technically significant at all. With respect to 4-octylphenol (and the related octylphenolethoxylates), the UK's development of a risk reduction strategy of 4-nonylphenol will include a targeted risk assessment of octylphenol and its derivatives. It is expected that the result will be an extension of measures provided for nonylphenol to cover octylphenol and its derivatives. What remains is the need to assess resorcinol and 4-nitrotoluene as a priority. The ICCA (International Council of Chemical Associations) programme on assessing priority substances is not suitable for evaluating the risks from endocrine effects, as the appropriate endpoints are not a requirement in the programme. However, the inefficiency of the European Existing Substances Programme should by no means be forgotten. Any possibility for speeding up the procedure should be exploited in the case of the industrial chemicals listed in Table 8 (see part I). To avoid delays, a targeted risk assessment, focusing on endocrine effects, should be initiated in cases where a comprehensive assessment is not expected in the foreseeable future.

**Excursion:** Are endocrinal effects *per se*, i.e. regardless of LOEC, hazardous?

In part I of this report, substance-related action targets are described, the second of which was: *"The irreversible input of xenobiotics with carcinogenic, mutagenic and reproduction toxic effects (CMR substances) into the environment must be avoided completely. This applies also to substances whose metabolites exhibit these properties."* Since such substances are capable of causing irreversible changes in organisms and ecosystems, a risk exists, regardless of the level of exposure, and this should be minimised. Scientific opinion is divided as to whether endocrine disrupters should also be included in this category: it is often pointed out that

endocrine mechanisms are generally triggered when a threshold dose is exceeded. However, Sheehan et al. were able to show that even very low doses of exogenic estradiol (and hydroxylated PCB), when applied to the eggs of the red-eared slider (trachemys scripta elegans), a turtle, could cause a gender shift, and no threshold value could be determined (Sheehan, 1999).[111] An already active system is being influenced, which would mean that endocrine disrupters should be treated like genotoxic substances, for example. Endocrine effects at low doses have been shown for only very few substances as yet. Completely abandoning the idea of comparing exposure and effect in describing risk is not sufficiently justified in the case of endocrine disrupters. There is currently good reason to examine effects at low doses especially thoroughly.

The following arguments also favour particular care when assessing endocrine mechanisms:

- Hormonally transmitted effects are frequently especially pronounced in certain stages of life, e.g. prenatally. This has not necessarily been considered in the tests conducted to date.
- There are indications that the relationship between concentration (dosage) and effect does not always rise monotonously in the case of endocrinal effects, i.e. effects can be found at concentrations below those where there was no effect (U shaped curve). Such relationships are not uncommon in pharmacology. Nonetheless, there are few data as yet to confirm this type of chemical effect. It is assumed that U shaped curves are due to multiple mechanisms such as homeostasis, or compensatory or protective reactions being activated.[112] The effects of TCDD on the thyroid gland and estrogenic activity of bisphenol A are examples of this form of dose-effect curve.

- Synergistic effects, i.e. mutually reinforcing, rather than merely additive, effects could be widespread among endocrine disrupters, although McLachlan's[113] withdrawal means that there is no conclusive evidence that endocrinel effects are significantly different to other types of effect. Nevertheless, additive effects are likely where the mechanisms are identical, which means that even substances at below their LOEC can contribute to an overall effect.

In view of our currently sketchy knowledge of endocrinal mechanisms, the following differentiated approach to assessment is appropriate,:

- Substances which are both hormonally active and persistent present a hazard when discharged into the environment, regardless of exposure levels. In view of the uncertainty of assessments, persistence in the environment and long-term consequences cannot be ruled out. Corresponding action targets in part I concerning the characteristic combinations

persistent/bioaccumulating and persistent/highly mobile are supplemented by this approach.

· Substances which are hormonally active but not persistent may be hazardous, depending on the measured or calculated exposure. The open questions in assessment do nevertheless give good cause for using greater margins of safety (MOS) when deriving PNEC or TDI values.

There is currently insufficient evidence to justify a total abandonment of exposure-effect comparisons, as in the case of genotoxic substances.

The assessment schemes described above will be proposed in further discussion of European assessment guidelines (e.g. TGD).

### *4.3 Hormonal pharmaceuticals*

Hormone-based medication is discharged into the environment, just as xenohormones are, especially estrogens, which are prescribed to humans and animals as contraceptives or hormone therapies.

The basic issue of environmental discharges of drugs has received increasing attention in recent years. Pharmaceuticals are appearing in concentrations of c. 0,01 µg/l up to over 1 µg/l in sewage treatment plants and small tributaries, synthetic estrogen 17α-ethinylestradiol in particular has been found in treatment plant effluents and surface water, as well as in sewage sludge and fish. The 51$^{st}$ German Conference of Environmental Ministers in 1998 decided that pollution by drugs and the key discharge paths should be investigated in a nation-wide testing programme, run by the individual *Länder*. The programme will probably cover three natural and three synthetic estrogens.

There are currently no representative data on the quantities of hormone-based medication prescribed to humans and animals. The quantities of 17 α-ethinylestradiol prescribed in Germany are estimated to be no more than c. 50 kg/a.[114]

Collating measured concentration data on estrogenic medicinal agents in sewage treatment effluent provides an average level of c. 1 ng/l; a maximum of 70 ng/l was found for estron, a natural estrogen.

In a study covering 15 surface water systems, only estron, at a level of max. 1.6 ng/l, was found.[115] The as yet not fully published study of the effects of estrogenic substances (17β-estradiol, ethinylestradiol) by the Fraunhofer Institute in Schmallenberg, part of multigeneration tests at significant concentrations has produced the following results for reproduction in *Danio rerio* (zebra fish): while concentrations of ethinylestradiol had practically no effect on embryo survival, hatching or gender ratios, a significant fall in the rate of fertilisation, due to disruption of physiological processes in male animals, was observed at 1.1 ng/l. An NOEC of 0.3 ng/l was determined for this most sensitive endpoint identified.[116]

From these preliminary results we can conclude that the relatively low concentrations of ethinylestradiol, only a few ng/l, are frequently already above the NOEC observed above for reproduction in fish. It is therefore likely that the effects detailed in Chapter 3 will be clearly influenced by steroids, even if the effect is not yet quantifiable. It is also to be expected that different organisms will react very differently. For example, female goldfish excrete the estrogen 17α, 20β-dihydroxy-4-pregnen-3-on into water to attract males (Urich, 1990).[117]

Based on current environmental regulations for licensing pharmaceuticals, no emissions reduction measures can be expected in the medium term, at least for human medicines, because regulation would be impracticable, or because revoking the licences of contraceptives or hormone therapies for environmental reasons would be unrealistic. It is more realistic to look at changing and developing the forms, dosages, etc. of environmentally hazardous drugs, in order to reduce discharges to the environment. Another avenue to explore is whether developing and using modern waste water purification technologies could eliminate hazardous substances more effectively.

### *4.4 Phytoestrogens*

Phytoestrogens occur naturally in plants, via which they can be ingested. Vegetable estrogens can be split into two substance groups: flavonoids (e. g. genisteine, coumestrol etc.) and lignanes. Soya products, a common source of protein in the foodstuffs industry, including milk substitutes in baby foods, are by far the largest source of vegetable estrogens.

It is known that some phytoestrogens, e.g. isoflavons, have an anticarcinogenic, i.e. positive, effect on human health. Until recently, little was known about the effects of human exposure to phytoestrogens such as coumestrol or genisteine as foods during pre- and perinatal development or childhood. A new study[118] has now shown that the sons of women who kept to a vegetarian diet during pregnancy have five times the risk of being born with genital malformation than the sons of women with a mixed diet. The authors take these findings as

reinforcement for the hypothesis that phytoestrogens have harmful effects on the development of male genitals. Further study in this area is urgently required.

Many agricultural cases have been documented, where wrongly mixed livestock feed or free-range grazing has led to considerable losses due to excessive consumption of phytoestrogens. For example, female sheep in Australia and New Zealand, Finland and in Israel are known to have suffered reproductive harm which was traced back to feeding on plants with a high phytoestrogen content (e.g. red clover). How these data relate to humans in still unclear.

The more active mating period which sets in after livestock which have been kept in stalls during the winter begin grazing, the higher rate of conception and lactation, are thought to be encouraged by phytoestrogens in certain legumes and grasses. On the other hand, cabbage and marsh horsetail lead to fertility disorders in female cattle if used as feed for lengthy periods.[119]

The public debate on risk assessment for endocrine disrupters in the environment often makes reference to daily consumption of phytoestrogens. Some scientists maintain that the quantities and effects of endocrine disrupters in the environment can be ignored when compared to phytoestrogens (on the basis of their relative endocrinal activity *in vitro*). It should be noted that a comparison of the relative estrogenicity *in vitro* is insufficient for a quantitative risk assessment of environmental endocrine disrupters and phytoestrogens. Exposure must be examined in more detail, and the quantity consumed or adsorbed is merely one variable in the equation. A comparison of *in vitro* potency and daily consumption rates alone is therefore scientifically meaningless.

A risk assessment must consider the necessary *in vivo* exposure and effect data, which can vary widely. This makes universal statements about entire substance groups impossible, especially since important aspects of exposure to and the effects of phytoestrogens are themselves heterogeneous.

A comparative assessment must consider the following exposure factors: sources and incidence in the environment, exposure paths, quantities absorbed, concentrations in the environment and in organisms, decomposition, bio- and geo-accumulation.

As for effects, studies should consider the strength and mechanism of the effect, bioavailability, metabolisation, paths and rates of excretion, storage and toxicodynamics. The major arguments against a simple comparison of the risks from xenoestrogens to phytoestrogens are:

– In contrast to certain industrial chemicals, there is no evidence for geo- or bio-accumulation of coumestrol or genisteine. On the contrary, they are metabolised very easily.

– In respect of decomposition/degradation, there is no evidence that phytoestrogens are environmentally persistent, in contrast to many endocrine disruptering chemicals.

– The mechanisms by which phytoestrogens and xenoestrogens function are not always the same, and therefore even comparing concentrations in the body is inadequate, due to their varying degrees of binding to hormone receptors.

As far as is known today, however, the possibility that phytoestrogens appear in food and the environment within the range of effect concentrations cannot be ruled out. This is especially true for people with special diets (e.g. vegetarians who eat a lot of soya products) or whose work exposes them to phytoestrogens (e.g. hops pickers). Also, detrimental effects in habitat-bound aquatic organisms, triggered by significant concentrations of estrogens in the waste water from pulp works, for example, have been well documented.

To sum up, the incidence and effects of phytoestrogens in the environment and in foodstuffs require further careful observation, but assessing the estrogenic potential of xenobiotics by way of a simple comparison of their effects with those of phytoestrogens is not scientifically meaningful.

## 5. Measures

In part I, proposals for structuring EU chemicals assessment and management more efficiently and orienting it more closely around the precautionary principle. The following aspects are particularly important for endocrine disrupters:

- Where a suspicion is sufficiently well-founded, the necessary regulations should be introduced, temporarily if necessary, even where some questions remain open.

- The substitution requirement under § 16 par. 2 GefStoffV (Hazardous Substances Ordinance) should be extended to cover environmental risks.

- “Blacklists” of especially critical substances which are not yet the subject of regulations should be used to inform users, consumers and the general public.

- Basic data on substances whose production/sales volume exceeds 1000 t/a (subsequently also at lower sales volumes) are to be made available at predetermined times. They should include any significant information on endocrine effects. (This should also be required for biocide products, under EU Regulation 1896/2000).

- Where possible, substances should be grouped by structure-activity relationships, to enable assessment and any subsequent measures to apply for the group as a whole.

- Meaningful data on exposure, including that of downstream users, are required.

- Monitoring programmes with international participation should be used to study chemical pollution, including that from endocrine disrupters.

- The basis for assessments, including those of endocrine effects, should be developed further.

### *5.1 General measures (not applying to particular substances)*

These principles, as well as the aspects outlined above, point to the following as priorities:

- Validation and further development of test guidelines to identify endocrine effects: the OECD's standardisation of test guidelines is a major indispensable step in being able to test substances systematically for any dangerous characteristics in respect of endocrinal potential. Complementing the currently discussed procedures with methods for studying invertebrates is a priority.

- Including such testing requirements in the licensing procedure for new substances and the authorization procedure for crop protection agents and biocides.

- Examining the numerous substances for which *in vitro* tests have provided evidence of endocrinal activity with valid *in vivo* tests is a priority. Levels of exposure, persistence and toxokinetic information should be considered in setting priorities (N.B. Even today, before the conclusion of the OECD's standardisation efforts, valid *in vivo* tests are available, to confirm or refute suspected endocrine effects).

- Reconsidering and developing the principles for assessing industrial chemicals (TGD) and crop protection agents. Especially important would be an agreement on greater margins of safety for endocrine effects (proposal: an additional factor of 2 to 5). If the

substances are also persistent, the long-term hazards make substitution essential in all open applications.

- Substances whose endocrine potential has been shown in *in vivo* tests, but where the available data is (as yet) insufficient for legal restriction or prohibition, should be named publicly in "blacklists", and made subject to a substitution requirement under § 16 par. 2 GefStoffV (Hazardous Substances Ordinance). Such a list could provide sufficient incentive to substitute, even where there is only a suspicion of danger. It should also be considered whether a hazard label for endocrine disrupters should be introduced.

- Existing analytic chemical monitoring programmes should be developed so as to gather representative data on exposure to endocrine disrupters.

- To identify the total pollution of ecosystems by endocrine disrupters, the analytic chemical monitoring programmes should be complemented by biological monitoring of organisms (e.g. gender ratios, vitellogenin content) and chemical/biological combination methods (effect-specific analytics).

- To assess effects on humans, a perinatal archive (lactate, placenta, umbilical blood) should be built up in the Environmental Specimen Bank, in order to be able to determine later the levels of contamination of substances which are not yet recognised as having an endocrine effect, and in order to interpret data on any effects.

- A national register of malformations should be built up within the Action Programme on the Environment and Health (APUG).

## *5.2 Substance-specific Measures*

### *5.2.1 Pesticides and Biocides*

In general, the environmental impact of *crop protection agents* is assessed most thoroughly. Under § 15 par. 1. 3 PflSchG (Crop Protection Act), endocrine effects must explicitly be included in the tests for unacceptable effects as part of the permissions procedure for crop protection agents, and the likelihood is correspondingly high that a permissions application will reveal any reasonable cause for concern about endocrine effects. The OECD test guidelines targeted at endocrinal activity, which will soon be available, should be incorporated into the test programme. Many of the substances listed in Chapter 4.2 are no longer permitted in Germany. Those which are still permitted are being assessed in the European Active Agent

Programme, which should pay particular attention to endocrine effects, and should study especially those which frequently appear in surface waters at concentrations above the targets.[120] Nonetheless, in most cases, other endpoints are more sensitive than the endocrine activity (even when increased safety factors are considered). Therefore, endocrine activity will generally not be decisive for the permission for certain applications. According to the currently available data, this (still) applies to triphenyltin as a fungicide for potato farming, but further testing is necessary.

The scope of the required tests for *non-agricultural biocides* has not yet been finally determined in the EU Biocidal Products Directive (98/8/EC). In any case, care should be taken to include tests with endpoints covering endocrine effects. The most important substances here are tributyltin compounds, especially in antifouling paints. The goal should be a complete ban, and stringent restrictions on tributyltin in dibutyltin compounds using Best Available Techniques, at first within Germany According to an expert hearing, conducted by UBA and BgVV in March 2000,exposure to tetrabutyltin, an intermediate in the production of tri-, di- and monobutyltin compounds, is not relevant. High concentrations have only been measured in suspended matter and in sediment in the Mulde, immediately downstream from a production site.

### *5.2.2 Pharmaceuticals*

As drugs are extensively tested before being licensed, their endocrine effects (not only of hormone treatments) are known, although the tests are restricted to humans and mammals. Regarding the assessment of the environmental effects of veterinary medicines, in which the UBA participates, generally no data on the endocrine effects are presented. In the absence of European technical guidelines, the environmental risks from human medicines are not assessed, a situation which is clearly to remedied. Also, data on the quantities and forms of administration of human and veterinary medicines is to be collected, and there is also a coordinated federal/Länder programme to study the incidence of medicines in the environment. Hormone medicines are part of this programme, and it is to be hoped that their impact to ecosystems can be better understood in future.

### *5.2.3 Industrial chemicals*

For new substances, no test results permitting conclusions about endocrine effects are presented in the base set, according to the concept of tonnage thresholds. Only for Stage 1 and onwards data is presented which may provide indications of such effects. As soon as the standardisation and validation of the OECD guidelines for testing endocrine effects is

completed, they should be included, so that effects can be recognised and clarified, at least starting from Stage 1.

There is generally a serious lack of data on the effects and behaviour of Existing substances (see part I). If the currently available data points to a substance being suspected of endcrine effects, and if this could influence the final decision, the remaining issues can be clarified with further tests, which must be completed by manufacturers within a certain period. If the results are not presented by then, or if further tests confirm suspicions, the substances should be published in a "blacklist", accompanied by a request to users to voluntarily refrain from using them.

The great majority of the industrial chemicals listed in Chapter 4.2 are mentioned in one of the four priority lists of the European Existing Substances Programme. The existing findings are included in the risk assessments, but they are only decisive if other effects are less sensitive. With a few strictly limited exceptions, substances evaluated on a European level are not subject to national regulation, and Germany's contribution should therefore be to draw attention to endocrine effects in risk assessments and to push for rapid implementation of any necessary risk reduction measures. If unreasonable delays occur, Germany should – if necessary in co-operation with other EU Member States – use the possibility of domestic regulation to accelerate measures at EU level.

4-octylphenol, 4-nitrotoluol and resorcinol are not mentioned in the EU existing substances programme. 4-nitrotoluol and resorcinol should immediately be included in the programme, as a matter of urgency, and undergo a targeted risk assessment under § 12 (2) Existing Substances Regulation 793/93/EC, to clarify the potential for endocrine effects and assess levels of exposure. Manufacturers should be asked to refute the initial suspicion without delay, that there are environmental hazards which have to be reduced, in order to avoid temporary European or – if the European procedure gets bogged down – domestic restrictions. 4-octylphenol should be considered in conjunction with other alkylphenols and alkylphenolethoxylates (see below).

Already in 1997, the UBA proposed domestic measures under § 17 ChemG (Chemicals Act), to deal with a*lkylphenols* and *alkylphenolethoxylates* (alkyl = butyl/C4 to nonyl/C9), as a significant environmental risk still exists, despite a voluntary commitment to reduce the use of these substances in washing and cleansing agents, and the conclusion of a risk assessment of only 4–nonylphenol was not then in sight. In August 1999 (BT-Drucksache 14/1471), the German Bundestag asked the government to review the washing and cleansing industry's voluntary commitment on the use of alkylphenolethoxylates, to search for a complete solution to the issues involving alkylphenols and alkylphenolethoxylates, and to implement the

necessary bans and restrictions nationally in order to speed up action at EU level. Due to the voluntary commitment, the consumption by the washing and cleansing industry of APEO had fallen by c. 90 % relative to the 1980s, marking a great success. Nevertheless, considerable remaining quantities (over 100 t/a) continue to be traded, especially by foreign companies or those who are not members of the German industrial associations, and further measures are required to guarantee the success of the agreement in the long term. After a hearing by the UBA in June and December 1998, it was also determined that the majority of APEO discharges into surface waters are diffuse discharges caused by product use. However, in the absence of a product register, quantifying the emissions at this level is not possible. The European risk assessment of 4-nonylphenol has now concluded that there are environmental risks in numerous areas of application (although this is based on the high toxicity to daphnia – which can have different reasons – and not on the potential for endocrine effects). The UK is currently developing a risk reduction strategy. In contrast to 1997, nonylphenolethoxylates and their degradation products (especially NP1EO and NP2EO) are now also being considered, although alkylphenol(ethoxylat)es with short alkyl chains (C5 to C8) are not. It was nevertheless decided that the UK should, while developing the risk reduction strategy, undertake a targeted assessment of 4-octylphenol(ethoxylat)es, raising hopes of speedy action at EU level. If these hopes should prove premature, the decision by the Bundestag requires action to be taken domestically. The significance of alkylphenols with even shorter chains should be examined more closely. One outcome of the June 1998 hearing is that less 4-tert-butyl- and 4-tert-amylphenol is being processed to ethoxylates, but rather being used in plastics production (e.g. for phenol resins and paints). The industry should be asked to present meaningful data on this development (N.B.: the inclusion of decylphenol in the substance list for the water framework directive is currently being discussed).

Alkylphenol(ethoxylat)es illustrate perfectly the limitations of the European chemicals programme, which operates on the basis of single substances, and which thereby tends to underestimate the risks. Alkylphenols and their ethoxylated derivatives appear in and affect the environment in combination with one another, and risk assessments should therefore try to consider whole substance groups. The concentration additive model is suitable for such cases,[121,122] and is to be developed for implementation in the current revision of the TGD.

In the case of alkylphenol(ethoxylat)es, Germany must - as well as actively taking a critical stance on EU activities and pushing for rapid decisions::

- Check the extent to which additional domestic measures are required for short-chained alkylphenols (butyl to heptyl) – possibly even decylphenol –(if necessary, a ban under § 17 ChemG, Chemicals Act).

- Check whether using an assessment based on *substance groups* (concentration additive model) for nonyl- and octylphenol reveals a greater need for action than the current consideration of *single substances*.

In recent years, the scientific community has been (sometimes bitterly) debating whether the low-dose effects of *bisphenol A* found by the vom Saal working group (premature puberty, reduced sperm production, inflation of the prostate, behaviour disturbance in mice), at concentrations of only a few µg/kg kg bw are reliable enough to form the basis of a risk asessment.[20,123,124,125] Ibrahim Chahoud's team at the Institute for Clinical Pharmacology at the FU Berlin[126,127], as well as a research team in the US[128], have now also been able to demonstrate effects on the male and female offspring of rats at low dosages (20 to 100 µg/kg kg bw). Tests conducted on *Marisa cornuarietis* by Oehlmann indicated effects at the surprisingly low concentration of under 1 µg/l.[129] There is also great uncertainty as to levels of exposure. Information from manufacturers produces an unclear picture of where the substance is used or emitted and of the associated risks. The EU draft risk assessment from the UK (not yet considering the above mentioned endocrinal results) points to environmental risks and a need for action in a number of areas of application. The UBA has examined patterns of exposure to bisphenol A more closely in interviews with industry members, finding that key areas are use in thermopaper and as antioxidant or oxidation inhibitor in PVC. The voluntary agreement announced by the European PVC industry in March 2000 contains no commitments on bisphenol A. Other unknown applications are to be examined in agriculture, as the substance has been found in manure, although this result must be confirmed by further analytical data. The following is recommended for this substance, a matter of debate for many years:

- Rapid preliminary conclusion of the EU risk assessment, with the goal of taking immediate risk reduction measures in those areas where a PEC/PNEC ratio > 1 already exists, and where clarification of unanswered questions is not in view (see the RAR draft of June 2000).

- Active participation in a research programme aimed at clarifying the open questions on low-dose estrogenic effects (both of invertebrates and vertebrates).

- Reviewing preliminary European risk assessments when comprehensive data on exposure and effects in low-dose levels become available.

# 6. Literature


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