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OPINION OF THE SCIENTIFIC COMMITTEE ON TOXICITY, ECOTOXICITY AND THE ENVIRONMENT (CSTEE) ON

"Two study reports on endocrine disrupters by WRc-NSF and BKH Consulting Engineers"

(WRc-NSF Ref: UC 6052; BKH Ref: M0355037)

Adopted by the CSTEE during the 40th plenary meeting of 12-13 November 2003

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Terms of Reference

The Committee on the basis of the following reports:

1. "Scientific evaluation of 12 substances in the context of the endocrine disrupters priority list of actions", carried out by WRc-NSF (UK).

2. "Endocrine disrupters: Study on gathering information on 435 substances with insufficient data" carried out by BKH Consulting Engineers (NL).

Is to answer the following questions:

WRc Study:

a) *The CSTEE is requested to assess the overall scientific quality of the WRc report. In considering this, the committee is asked to comment on the source material, and on the validity of the methodology and assumptions used to assess the effect of the substances evaluated in the study*

b) *Does the CSTEE agree with the conclusions presented in the report? If the CSTEE disagrees with the conclusions or any reasoning, the CSTEE is invited to elaborate on the reasons for this divergence of opinion.*

BKH Study

a) *The CSTEE is requested to assess the overall scientific quality of the BKH report. In considering this, the committee is asked to comment on the source material, methodology and selection criteria used by the authors for classifying specific substances as potential endocrine disrupters*

b) *Does the CSTEE agree with the conclusions presented in the report? If the CSTEE disagrees with the conclusions or any reasoning, the CSTEE is invited to elaborate on the reasons for this divergence of opinion.*

c) *Does the CSTEE consider that the preliminary evaluation of substances presented in this report provide an appropriate scientific basis on which to establish a priority list of substances for further evaluation of their role in endocrine disruption? If not, what other approach or methodology would the CSTEE suggest?*

Context

In June 2000, the Commission established a first priority list of substances for further evaluation of their role in endocrine disruption (study report entitled: "Towards the establishment of a priority list of substances for further evaluation of their role in Endocrine Disruption – preparation of a candidate list of substances as a basis for priority setting" carried out by BKH Consulting Engineers, NL). In a first step, a candidate list of 553 substances was identified, from which evidence of endocrine disruption or potential endocrine disruption was found for 118 substances. An analysis of the legal status of these 118 substances revealed that 9 substances were neither restricted nor being addressed under existing Community legislation.

On basis of the examination of this report carried out at the request of DG ENV, the CSTEE came to the following conclusions in its opinion of 5 September, 2000:

a. Are the source material, methodology and selection criteria used to include substances in the consultants' list logical and scientifically relevant?

The CSTEE supports a stepwise approach for the selection of substances for further evaluation and prioritisation. However, the CSTEE does not find the source material, methodology and selection criteria used to be scientifically adequate.

b. Does the CSTEE consider that the preliminary evaluation of substances presented in the study report is an appropriate scientific basis on which to establish a priority list of substances for further evaluation of their role in endocrine disruption? If not, what other methodology and/or evaluation would the CSTEE suggest, bearing in mind the context described above?

The Committee concludes that there are important shortcomings in the present approach. Especially, this relates to the omission of dose-response/potency considerations and the inclusion of too restrictive persistence and production volume criteria in the second step of the selection process. Further, more quantitative exposure information should have been included in the second step. Another important omission is that synthetic hormones released into the environment have not been considered. Also, substances lacking data have not been properly addressed. Several suggestions for improvement of the current approach are presented in the comments to the study report.

In June 2001, the Commission adopted a follow up Communication to the Council and European Parliament on the implementation of the Community Strategy for Endocrine Disrupters [COM (2001)262]. In this Communication (EC 2001) the Commission proposed a priority list of actions to further evaluate the role of specific "candidate" substances in endocrine disruption, after consultation with stakeholders and scientific committees in the Commission.

One of these priority actions was the initiation of an in-depth evaluation of a group of 12 candidate substances consisting of 9 industrial substances (2,2'-bis(4-(2,3-epoxypropyl)phenyl)propane, carbon disulphide, 4-chloro-3-methylphenol, 2,4-dichlorophenol, 4-nitrotoluene, o-phenylphenol, resorcinol, 4-*tert* octylphenol and 2,2',4,4'-tetrabrominated diphenyl ether or tetraBDE) and three natural/synthetic hormones (oestrone, oestradiol and ethinyloestradiol). This evaluation considers up-to-date ED evidence, including dose-

response/potency/timing/synergy considerations, comparison with normal toxicity data, and quantitative exposure assessment where appropriate. Exposure assessment would include the identification of specific cases of consumer or ecosystem exposure which might warrant special consideration in the short-term. In addition, the three synthetic/natural hormones should be evaluated in order to gather up-to-date evidence of environmental exposure and effects related to these substances.

At the same time, it was decided to give equal priority to gathering data/information on persistence, production volumes and legal status on another 435 candidate substances for which there was insufficient data in the BKH 2000 report, to decide on endocrine disruption or potential for endocrine disruption (due not to lack of data but to lack of resources to gather the data).

Thus in 2001, the two studies were launched simultaneously. The first, on the 12 substances identified, was carried out by WRc-NSF (UK). The second one, on 435 substances, was carried out by BKH Consulting Engineers (NL).

- On January 8, 2002 a CSTEE working group meeting was held with presentations by representatives of BKH and WRc on the studies they had undertaken at DG ENV's request. In particular, there seemed two items worth exploring: (i) the evaluation of data on endocrine disruption - so-called weight of evidence and (ii) the methodology by which to take a "second cut" of data from 435 substances.

- The working group had found the presentations very useful, noting that the previous criticisms of the CSTEE had been thoroughly addressed. A number of specific questions and comments had been given to the presenters. The CSTEE concurred with the position of the working group (see minutes CSTEE meeting 9 January 2002).

In a second step, the Commission will decide, in consultation with stakeholders, on the scope, contents and presentation of this new priority list of substances for further evaluation of their role in endocrine disruption.

Before the Commission decides on this new priority list, the CSTEE is invited to give its opinion on the scientific soundness of the two study reports by WRc-NSF and BKH Consulting Engineers.

Summary Opinion

WRc Study:

a) The CSTEE is requested to assess the overall scientific quality of the WRc report. In considering this, the committee is asked to comment on the source material, and on the validity of the methodology and assumptions used to assess the effect of the substances evaluated in the study.

The CSTEE is in agreement with the overall scientific approach of the report and finds the developed evaluation framework appropriate. The report is well-structured and reflects state-of-the-art knowledge regarding the compounds evaluated. Assessment of the data and methodology, and assumptions used to evaluate the effects of the eleven compounds are sound. The CSTEE notes, although it agrees with the overall conclusions for the various compounds,

that the underlying data for 2,4 dichlorophenol and the three oestrogens sometimes contain errors or are incomplete e.g. limited notice is given to *in vitro* studies.

b) Does the CSTEE agree with the conclusions presented in the report? If the CSTEE disagrees with the conclusions or any reasoning, the CSTEE is invited to elaborate on the reasons for this divergence of opinion.

The CSTEE agrees with the overall conclusions regarding endocrine disruption effects of the report, but after detailed assessment of the individual compounds disagrees with the conclusion for 2, 4-dichlorophenol with respect to wildlife. Considering the very limited data set on the environmental effects of this compound, the CSTEE is of the opinion that, based on the data in the report, no conclusions can be taken on the risks posed by this chemical to the environment.

BKH Study:

a) The CSTEE is requested to assess the overall scientific quality of the BKH report. In considering this, the committee is asked to comment on the source material, methodology and selection criteria used by the authors for classifying specific substances as potential endocrine disrupters.

The BKH report provides a significantly improved assessment in comparison with the earlier report. In general, the CSTEE agrees with the methodology and selection criteria that have been used. The CSTEE notes that available data on endocrine disrupter effects especially for pesticides have not been used to any great extent. Several chemicals are plant protection products and a comprehensive risk assessment is conducted under Directive 91/414/EC. The use of this information has been very limited, thus the CSTEE considers that this information should be assessed. Regarding individual chemicals only in very few cases industry provided data.

b) Does the CSTEE agree with the conclusions presented in the report? If the CSTEE disagrees with the conclusions or any reasoning, the CSTEE is invited to elaborate on the reasons for this divergence of opinion.

For the most part, the CSTEE agrees with the conclusions presented in the report. However, it is misleading to derive endocrine disrupter potency ratios by comparing endocrine disrupter effects for non-oestrogenic chemicals with the oestrogenicity of 17ß-oestradiol, since endocrine disrupter activity may involve non-oestrogenic mechanisms.

c) Does the CSTEE consider that the preliminary evaluation of substances presented in this report provide an appropriate scientific basis on which to establish a priority list of substances for further evaluation of their role in endocrine disruption? If not, what other approach or methodology would the CSTEE suggest?

Overall, the CSTEE finds that the scientific preliminary evaluation provides an appropriate basis. However, available toxicity and ecotoxicity data on pesticides can be used directly rather than being inferred. Also, low production volume chemicals but with high release into the environment or of high potency, are not sufficiently covered in the report.

It is emphasised that the prioritisation should be an iterative process so that new information is evaluated when it becomes available. This is of particular importance as the database of most compounds on endocrine disruption is limited, especially in the field of ecotoxicity.

WRC REPORT

Evaluation of the reviewed compounds

In the evaluation of the compounds critical endocrine disruption effects are used, and based on an earlier opinion of the CSTEE (2000): "Only in situations where the endocrine disrupting effect is critical (i.e. is the most potent) in comparison to other toxic effects, should the endocrine disruption be considered for hazard and risk assessments."

2,2-Bis(4-(2,3-epoxypropyl)phenyl)propane (BADGE)

Human health:

Toxicological issues of BADGE are mostly related to a possible mutagenic activity. Due to a weak DNA binding capacity, when applied directly to mouse skin and the resulting concern about the occurrence of a possible similar mechanism in the gastrointestinal tract, a 2 year-gavage chronic toxicity/carcinogenicity study has been launched by industry. Results should be available by February 2004.

In studies that have been done to assess the carcinogenic activity of BADGE, there is no report of a biological response indicative of endocrine mediated changes such as an increase in the incidence of mammary gland cancers.

In a sub-chronic oral gavage study rats were exposed to 0, 50, 250 and 1000 mg/kg body weight. No effects were found on endocrine organs and tissues, with the exception of testis and uterus at the 1000 mg/kg level resulting in a NOAEL for endocrine effects at 250 mg/kg. However, in this study general systemic toxicity effects (alterations in body weight and serum cholesterol) were noted at the 50 mg/level indicating that the observed endocrine effects may have resulted from direct toxic action.

In one- and two-generation reproduction studies in rats exposed orally to BADGE no effects on reproductive parameters occurred at the highest dose levels tested (540 and 750 mg/kg body weight per day).

In developmental studies in rats and rabbits no foetotoxic or developmental effects were noted at the highest dose level tested (300-540 mg/kg body weight per day); maternal toxic effects occurred at lower levels. However, in a dermal teratogenicity study in rabbits, a decrease in the pregnancy rate and a change in the foetal sex ratio were found at a dose of 30 mg/kg, although no effects relative to the controls were seen at the 100 and 300 mg/kg/day doses. The report agrees that these effects can be considered as random events in view of the lack of a monotonic dose response relationship.

In an uterotrophic screening assay, subcutaneous injections of doses up to 1 mg/kg for 3 consecutive days did not result in weight and histology changes of uterus and vagina indicating no oestrogenic response of BADGE.

In vitro studies indicate an absence of induction by BADGE of oestrogen-sensitive gene products and no or weak binding to the human oestrogen receptor. In a single assay (only abstract available) a slight binding to the androgen receptor was reported.

No TDI has been set so far but the Scientific Committee on Food has recommended an upper limit of 1 mg/kg food as a temporary guidance level. Taking into account a daily consumption of 2-3 kg of food per day, a daily maximally tolerated exposure of 3 mg/person can be assessed. Given both its use as a food additive and the concerns arising from its migration from can-coating, contaminated food represents the major source of human exposure. In this context, BADGE is regulated by numerous Directives: 90/128/EEC, 2001/61/CE 2002/16/EC.

Environment:

Given its uses and its physico-chemical characteristics (very poor solubility in water, low volatility, short half-life and rapid metabolism to non-toxic metabolites), wildlife exposure to BADGE is likely very limited.

Since assays for aquatic toxicity have not been carried out under flow-through conditions and in the absence of data of monitoring of the water concentrations, only nominal concentrations are available. The significance and usefulness of the data are therefore more than questionable. The relevance of the data for aquatic organisms toxicity should therefore be considered with great care, if not, erroneous conclusions may be drawn.

Regarding endocrine disrupter related effects, the only available study is a 21 day reproduction study on *Daphnia magna* from which a NOEC of 0.3 mg/L was derived with lethality as the endpoint. The CSTEE considers that the inclusion of the *Daphnia magna* reproduction test is not relevant to the assessment of potential endocrine disrupting effects as this test is conducted under parthenogenetic reproductive conditions, and therefore not able to respond to some key endocrine disrupter activities such as oestrogenicity, as clearly shown in studies conducted with synthetic and natural hormones.

Specific comments:

Water solubility of BADGE has been assessed contrary to what is written in table 3-1.

Conclusion:

The CSTEE agrees with the report that BADGE does not appear to cause critical endocrine disrupter effects in humans and wildlife.

Carbon disulfide

Human health:

The report is primarily based on the BUA report (1991), the IUCLID (2000) and the draft of the CICAD (IPCS 2002), describing that CS_2 the probably most critical endpoints are neurotoxicity and cardiotoxicity.

Whereas no *in vitro* studies on endocrine disrupter effects are reported a number of repeated dose studies in laboratory animals indicate the potential to affect hormonal sensitive organs and

tissues. In humans several reports in workers on gonad and hormone effects in males and hormone and pregnancy effects in females have been reported.

It is concluded that the NOAEL for the most sensitive biological endpoints was between 150 and 800 mg/m^3 in animals (neurotoxicity and cardiotoxicity) and between 10 and 60 mg/m^3 in humans for the same endpoints. NOAELs for effects on reproduction in laboratory animals (male fertility in rats through changes in sperm count and mating behaviour) were >1000 mg/m^3 and in humans for effects on changes in sperm morphology >30 mg/m^3. Embryotoxic effects (increased resorptions, skeletal and visceral abnormalities) have been seen in rabbits at 3798 and 1899 mg/m^3, the highest dose resulting in maternal toxicity. The NOEL was 949 mg/m^3. In another study the maximal concentration of 126.7 mg/m^3 was without effects. In a teratogenicity study in rats 1266 mg/m^3 showed maternal toxicity and embryotoxicity, whereas 633 mg/m^3 was without effects.

CSTEE agrees with the conclusion that the most sensitive endpoints of CS_2 is cardiotoxicity and neurotoxicity. This is supported by more recent studies on the cardiotoxic and neurotoxic effects of CS_2, that corroborate the specific sensitivity of the heart at and below 10 ppm occupational CS_2 exposure (Huang et al 2002, Krestev et al 2003, Korinth et al 2003) as compared to endocrine effects. In a six year prospective cohort study on 432 male workers exposed to CS_2 in rayon factories in Japan and 402 reference workers of the same factories, Takebayashi et al (2003) did not observe biological significant effects on thyroid, hypophysis, and gonad function at median or average exposure levels to CS_2 about or below 10 ppm (32 mg/m^3).

Environment:

There appear to be no *in vitro* studies addressing the endocrine disrupting effects of CS_2 in cells or tissues of wildlife, and there are very limited *in vivo* data addressing the potential endocrine disrupter effects on aquatic, terrestrial or aerial organisms. A data search did not reveal additional relevant data.

The report is referring to only three studies on aquatic species. In studies (Gate 1985, Van Leewen et al. 1986) of embryos of the frog *Microhyla ornata* and the fish (*Oncorhynchus mykiss*) malformations of the notochord were found at nominal exposure concentrations at or above 100 mg/l. A study by Akzo Nobel (1991) reported effects of CS_2 on the hatching rate of zebrafish. In this study groups of eggs were exposed to nominal CS_2 concentrations 0, 24, 76, 243 and 778 µg/l for 10 days in the first study and to 0, 1000, 2500 and 6250 µg/l for 8 days in the second study both under semi-static conditions and in closed vessels. In the first study no effects on the hatching of eggs and malformation and lethality of larvae were evident at any of the concentrations. In the second study effects were evident at the two higher concentrations. The most toxic endpoint was the hatch rate of the eggs. CSTEE agrees with the NOEC derived (NOEC 1 mg/l) and NOEC for specific malformations and survival (2.5 mg/l). The respective LOECs were 2.5 and 6.25 mg/l. However, it is not clear if these effects are endocrine mediated effects.

No information is available on endocrine disrupter effects of CS_2 on reproduction of aquatic organisms. The effect concentration in the zebra fish study is similar to the acute toxicities (LC50 2.1-4 mg/l) for fish (guppy; *Poecilia reticulata*) and aquatic invertebrates (*Daphnia magna*).

No data are available on the potential endocrine disrupting effects on the reproduction/development of terrestrial or aerial organisms. However, CSTEE suggests that the data from inhalation studies of laboratory mammals (rats and rabbits) can be used to assess risk for terrestrial mammals following inhalation exposure.

Since most CS_2 is released to air, it is the terrestrial and aerial organisms in the vicinity of industrial sources that are at the highest potential risk. Aquatic organisms close to discharge points may also be potentially affected. However, environmental exposure from releases from industrial sources is estimated to be low. Measured environmental exposure data are not available. The IUCLID database indicates that typical aquatic CS_2 concentrations were < 10 ng/l. Thus, the estimated exposure concentration in water is very low relative to no effect-levels in fish. The data on potential endocrine mediated responses in rats and rabbits following inhalation exposure (NOEL for embryotoxic effect > 600 mg/m^3) indicate that it is unlikely that CS_2 exposure causes adverse effects on populations of terrestrial mammals living in the vicinity of CS_2 releases to the atmosphere. Even though the database is very scarce for risk characterisation of CS_2 exposure of wildlife, CSTEE agrees with the conclusion that it is unlikely that CS_2 may cause adverse effects on populations of terrestrial and aquatic wildlife. However, it needs to be recognised that environmental concentrations of CS_2 are influenced by natural releases.

Conclusion:

The CSTEE agrees with the report that carbon disulphide does not appear to cause critical endocrine disrupter effects in humans and wildlife.

4-Chloro-3-methylphenol

Human health*:*

There are few reproductive toxicity studies available including both effects on development and fertility of 4-chloro-3-methylphenol, in order to elucidate a possible endocrine activity of this substance. The reported studies indicate that general systemic toxicity is induced at doses below the dose that induce effects on reproduction. However, most of these studies are performed at low doses. When considering a possible endocrine activity of 4-chloro-3-methylphenol, there are limited indications from *in vitro* studies with mammalian cells and tissues that 4-chloro-3-methylphenol might have a weak oestrogen activity.

The study from Bartmann (1991) is the only available developmental and teratogenicity study. In this study rats were exposed to 4-chloro-3-methylphenol between day 6 and 15 of gestation. To be able to detect endocrine disrupter effects, the animals should have been exposed until gestation day 20, since the last part of gestation is the most sensitive period for detecting a possible endocrine activity of a chemical. In this study maternal toxicity was reported from 100 mg/kg bw, and intra-uterine developmental toxicity from 300 mg/kg bw. No statistically significant increase in teratogenicity was reported at 300 mg/kg bw, the highest dose tested.

There are two 13 week (90 day) studies available. The first one is Eiben (1988) where 4-chloro-3-methylphenol was administered in the diet, and the second is Leser (1991) where 4-chloro-3-methylphenol was administered via the dermal route. However, in these studies adult animals were used, and since the most sensitive period for exposure to endocrine disrupters is

the perinatal period up to puberty, it is difficult to draw any conclusion from these studies related to a possible endocrine activity of 4-chloro-3-methylphenol.

There is no multi-generation reproduction study available for 4-chloro-3-methylphenol.

Overall, since the available studies have limitations in the detection of a potential endocrine activity of 4-chloro-3-methylphenol, a developmental toxicity study according to OECD Guideline 414 with exposure from gestation day 6 to 20 is warranted, or a 2-generation study according to the revised OECD Guideline 416. Information from such studies will be of importance in the evaluation of a potential endocrine activity of 4-chloro-3-methylphenol.

Environment:

No *in vitro* studies are reported and the *in vivo* studies are restricted to *Daphnia magna* reproduction assays. As mentioned previously the *Daphnia magna* reproduction test is not relevant for assessing potential endocrine disrupting effects. Thus, the information on aquatic species is restricted to general ecotoxicity studies.

Data suggest potential differences among taxonomic groups, although the variability within each group is also very high. The Acute-to-Chronic ratios are very low, being close to one even for the same species, although the endpoints measured in the 14 days fish study are not mentioned. These results could suggest specific mechanisms of action, but the relevance of endocrine disruption cannot be established without proper chronic data on fish and non-partenogenetic invertebrates.

The report suggests that available exposure data indicates low risk for aquatic organisms. However, this conclusion is based on the use of "standard" margins of safety, which have been recognised not to be acceptable for endocrine disrupting chemicals.

Conclusion:

The CSTEE agrees with the report that 4-chloro-3-methylphenol does not appear to cause critical endocrine disrupter effects for humans and wildlife, but as noted in the report there are considerable limitations in the database.

2,4-Dichlorophenol

Human health:

Several *in vitro* and *in vivo* studies addressing the toxicity of 2,4-dichlorophenol have been performed. The studies cited do not reveal adverse endocrine disrupting effects of the substance in the absence of maternal toxicity. However, several endpoints relevant for the evaluation of endocrine disrupting potential have not been properly addressed. In the two developmental/teratogenicity studies cited, the animals have been exposed until day 15. To be able to detect endocrine disrupter effects, the animals should have been exposed until gestation day 20, since the last part of gestation is the most sensitive period for detecting a possible endocrine activity of a chemical.

Most importantly, the review refers to an ongoing 2-generation reproductive study (MITI, 2002) and it is thereby advisable to await the results of this study before concluding on the endocrine disrupting potential of 2,4-dichlorophenol.

Specific comments:

- Page 7-9: In the one-generation study cited (Exon et al. 1984; Exon and Koller 1985), it is stated that the study was performed in a way which was consistent with OECD TG 415. However, in this study exposed females were mated to untreated males. This is not in accordance with OECD TG 415 that requires that both males and females should be exposed to the test compound.

- Page 7-10: It could be argued that the 74 mg/kg dose in the NTIS study (1968) represents a LOEL value and not a NOAEL value as effects on foetal mortality, foetal weights and limb extension are reported. The evaluation of maternal toxicity is difficult from the study description.

- Table 7.6: The NOAEL-value in study by Borzelleca et al. should be 500 mg/kg and not 50 mg/kg.

- Table 7.8: Repeated dose toxicity: the NOAEL value derived from the study by Kobayashi et al. should be 100 mg/kg and not 1000 mg/kg.

- It seems from the description of the 90 days NTP-study on page 7-19 that the correct NOAEL should be 5000 ppm and not 2500 ppm.

- Page 7-22: The statement in chapter 7.5.1.3 that foetuses are markedly more sensitive to 2,4-dichlorophenol than juveniles and adults does not seem warranted from the data cited in this review. It is also in contrast to the conclusion that foetal effects are secondary to maternal toxicity. It should be emphasised that the NOAEL value derived from the one-generation rat study (Exon 1984; Exon and Koller 1985) represents the highest administered dose, and that no endocrine disrupter effects were established from this study. From the data presented in the current review it does not seem possible to draw any conclusion as to differences in sensitivity to 2,4-dichlorophenol-toxicity in different age groups.

Environment:

There is no evidence from *in vitro* or *in vivo* studies suggesting a potential for oestrogenic effects. The CSTEE considers that the inclusion of the *Daphnia magna* reproduction test is not relevant for the assessment of potential endocrine disrupting effects as this test is conducted under parthenogenetic reproductive conditions, and therefore not able to respond to some key ED activities such as oestrogenicity.

The information for 2,4, dichlorophenol on aquatic species is therefore restricted to general ecotoxicity studies.

The studies conducted on fish include acute and chronic with several early life cycle studies including a 85 days flow-through study. An Acute-to-Chronic ratio close to 10 is observed, in

addition, even in the chronic studies, mortality seems to be slightly more sensitive than other parameters, including growth and development.

The toxicity values reported for aquatic invertebrates are in the same range than those reported for fish. Again an Acute-to-Chronic ratio close to 10 is observed. There is a single case of sublethal effects on larvae from the Genus *Hydropsyche* at very low concentrations. However, this is an effect also observed for other chemicals in this taxonomic group (e.g. Camargo et al., 1992) and should not be related to endocrine disruption, but to the sensitivity of the endpoint itself.

The CSTEE concludes that there are no evidences of endocrine disrupting effects, and the toxicological profile indicates that the effects are associated to generic mechanisms of action. The similarities in acute toxicity among taxonomic groups, the Acute to Chronic ratio around 10 and the relevance of lethality as endpoint suggest that endocrine disruption is not relevant.

The monitoring data are not consistent enough for supporting the conclusion of a potential risk.

Conclusion:

The CSTEE agrees with the report that 2,4-dichlorophenol does not appear to cause critical endocrine disrupter effects for humans, but as noted in the report there are considerable limitations in the database.

Regarding wildlife, the CSTEE agrees with the report that there are uncertainties with respect to potential adverse effects of 2,4-dichlorophenol on reproduction and development, especially in fishes, but notes in an overall assessment of the available data that, in contrast to the report, the concerns for endocrine disrupter effects is low.

4-Nitrotoluene

Human health:

The report correctly describes the *in vitro* studies, subchronic and chronic studies in animals and man which have been available by the end of the project.

It is concluded that toxicity studies in rats and mice have indicated that the most sensitive endpoint of 4-nitrotoluene toxicity is methaemoglobinemia, leading to anaemia, Heinz body formation, reticulocytosis and increased haematopoiesis with secondary effects on the spleen. These effects have been seen at exposure doses of 42 mg/kg bw per day in rats and 131 mg/kg bw per day in mice. In a two year carcinogenicity study, effects were evident on reproductive tissues such as germinal epithelial atrophy in the testes of male rats. However, the effect levels of the potential endocrine responses are higher, in the rat dietary carcinogenicity study > 110 mg/kg bw per day, in mice 660 mg/kg bw per day. As a result it is considered probable that the most sensitive toxic effects observed in mammals following exposure to 4-nitrotoluene are not endocrine mediated and that current exposure patterns do not present a risk to workers or consumers.

The IUCLID database from which all information has been taken indicated that there is an ongoing reproduction/development screening test (OECD 421) on 4-nitrotoluene. The results of this study became available recently (Bayer 2002).

12 male and 12 female rats per group received 0, 25, 100 or 400 mg 4-nitrotoluene/kg bw per day by gavage for 2 weeks before mating and thereafter (males for 35, females for 46 additional days, respectively). Additional investigations were examinations of liver, spleen, kidney, pituitary gland, uterus, uterine cervix and vagina, mammary gland, epididymis and prostate. Insemination index, fertility index and time to insemination were not affected by treatment up to and including 400 mg/kg bw/day. The gestation index was not affected up to and including 100 mg/kg bw/day. In the high dose the gestational index was marginally reduced because a dead litter was found in 1 female together with an increased prenatal loss of the remaining litters. On day 4 post partum mean body weight of pups were slightly reduced, but significantly in the highest dose group. The following No or Lowest Adverse Effect Levels were derived:

NOAEL general toxicity, males:	25 mg/kg per day
LOAEL general toxicity females:	25 mg/kg per day
NOAEL reproduction toxicity:	25 mg/kg per day
LOAEL reproduction toxicity:	100 mg/kg per day (reduced pup birth weight, at 400 mg/kg reduced litter size).

In the developmental toxicity part of the study clear signs of maternal toxicity were seen at 400 mg/kg, at 100 and 25 mg/kg. Feed intake and body weight gain during lactation were marginally reduced. 4-Nitrotoluene caused significantly reduced pup body weights at dose levels at which maternal toxicity was seen with:

NOAEL for developmental toxicity of 25 mg/kg bwt/day

LOAEL for maternal toxicity of 25 mg/kg bwt/day.

The study confirms previous observations that effects on reproduction and development occur at higher doses than effects on other targets.

Environment:

Two *in vitro* studies on the oestrogenic receptor are reported but results are not conclusive.

As mentioned previously the *Daphnia magna* reproduction test is not relevant for assessing potential endocrine disrupting effects. Thus, the information for 4-nitrotoluene on aquatic species is restricted to general ecotoxicity studies.

The studies conducted on fish indicate that lethality seems to be a poor parameter for describing effects. No information on the endpoints measured in the long-term tests on fish is provided, and therefore, no conclusions on the relevance of endocrine disrupter effects can be presented.

The report suggests that available exposure data indicates low risk for aquatic organisms. However, this conclusion is based on the use of "standard" margins of safety, which have been recognised not to be acceptable for endocrine disrupting chemicals.

Additional data, as suggested in the report, should be requested for investigating the high Acute-to-Chronic ratio observed for fish and clarify the potential role of endocrine disrupter effects.

Conclusion:

The CSTEE agrees with the report that 4-nitrotoluene does not appear to cause critical endocrine disrupter effects, but there are limitations in the data base for the environment.

o-Phenylphenol

Human health:

The report is well-structured, with a high level of detail in each of the sections, and with useful and adequate summaries for a quick overview.

The contents appear to reflect state-of-the-art knowledge regarding the substance under study. They give a good account of the relevant literature, which is described in sufficient detail and gives the reader an accurate overview of current science.

The critical study appears to be a well-performed two-generation study in the rat (Eigenberg 1990), which showed a NOAEL of 35 mg/kg bw/day based on urinary bladder hyperplasia and papillomatosis at 125 mg/kg bw/day. Pup weight effects in both generations occurred at 457 mg/kg bw/day, starting after lactation, indicating that lactational transfer did not play a major role in the effects found. It is concluded that endocrine mechanisms are unlikely to be important in the toxicity of o-phenylphenol.

Environment:

For studies of o-phenylphenol in aquatic invertebrates LC-50 values of 1.5 to 4.5 mg/l, for *Daphnia magna* and *Lymnea,* are reported. Fish studies indicate LC-50 values ranging from 2.8 to 6.2 mg/l.

Data on reproduction effects in wildlife are limited to *Daphnia magna* (a test that is conducted under parthenogenetic reproductive conditions that is not relevant for the assessment of potential endocrine disrupting effects) and fathead minnow (*Pimephales peomelas*) which showed reduced reproduction at NOEC values of 0.009 and 0.036 mg/l, respectively, levels that are lower than the threshold levels for general toxicity. In the fathead minnow no effects on vitellogenin induction were noted indicating that the effects on reproduction were not oestrogen mediated. It has been reported that o-phenylphenol was able to induce vitellogenin gene expression in rainbow trout hepatocyte cultures, but only at unrealistically high concentrations (17 mg/l.), and no mechanism of action for this effect was proposed.

As indicated in the report, the absence of data on concentrations of o-phenylphenol in surface waters precludes an assessment of the margin of safety relative to exposure concentrations causing endocrine mediated responses in aquatic organisms.

Conclusion:

The CSTEE agrees with the report that o-phenylphenol does not appear to cause critical endocrine disrupter effects, but as noted in the report there are limitations in the data base for the environment.

Resorcinol

Human health:

Various *in vitro* studies carried out between 1985 and 1995 indicate that resorcinol may induce alterations in the thyroid due to inhibition of peroxidase enzymes. In porcine thyroid gland slices, the uptake of iodine to form precursors of mono- and di-iodotyrosine was significantly inhibited. In another experiment porcine thyroid peroxidase was inhibited in the presence of resorcinol at concentrations around 0.3 µg/L. Similarly, resorcinol inhibited lactoperoxidase activity (which is closely related to peroxidase) but at a much more higher concentration (0.22 mg/L). *In vitro* studies with resorcinol did not show oestrogenic or anti-oestrogenic activities.

Old non-GLP *in vivo* studies (1985) had revealed reversible anti-thyroid activity. Recent NTP studies in rats and mice receiving up to 520 (rats) or 420 (mice) mg/kg bw/day by oral gavage in water for 13 weeks did not confirm these data. In these NTP studies changes in adrenal weight have been reported. Given the fact that there was an increase adenal weight in rats, a decrease in mice, no dose-effect relationship, positive at all doses tested, the biological significance of these findings is unclear.

As there are no data available regarding potential effects of resorcinol on reproduction and fertility, a Resorcinol Task Force is currently performing a study in the rat to examine the potential effects on the postnatal development of offspring. The CSTEE agrees with the report that this is a critical area of uncertainty, as is the adrenal toxicity observed in the NTP studies.

Environment:

The question of potential adverse effects of resorcinol on the reproduction and development cannot be answered given the absence of key data, especially for aquatic organisms which seems to be the only compartment of relevance when considering exposure of wildlife to resorcinol. This uncertainty is planned to be addressed by the Resorcinol Task Force.

The available data on the trout and zebrafish embryos show teratogenic effects at concentrations > 100 mg/L. These studies were conducted by exposing newly fertilized eggs to resorcinol for periods of 60 (trout) or 7 (zebrafish) days, the test solutions were renewed 3 times a week, but no analytical confirmation of the concentrations was performed which slightly impairs the validity of the study. There are discrepancies in the report regarding the available information on the stability of resorcinol in solution in water (table 3.1 p. 3.2 indicates that data on the stability in water are available, whereas in table 10.1 p.10.5 it is written that there is no data on the abiotic aquatic degradation, and on the top of p. 10-41, that "*resorcinol undergoes rapid degradation in water*", and finally, the report words (p. 10.28) on the reliability of the acute toxicity tests which are conducted for 24, 48 or 96 hours are : " *a number of these studies used a static exposure regime and did not measure the actual test concentrations which raises issues regarding the validity of the data*". This point needs clarification. In addition, there is no indication as to whether the effect is endocrine mediated or not.

Conclusion:

The CSTEE agrees with the conclusions of the report that further testing of resorcinol for potential endocrine disrupting effects is needed. This concerns both human health and the environment, issues addressed in the Resorcinol Task Group.

4-tert-Octylphenol

Human health:

The report is well-structured, with a high level of detail in each of the sections, and with useful and adequate summaries for a quick overview.

The contents appear to reflect state-of-the-art knowledge regarding the substance under study. The report gives a good account of the relevant literature, which is described in sufficient detail and gives the reader an accurate overview of current science.

A well-conducted extended 2-generation study was performed with 4-*tert*-octylphenol with additional parameters added that are not mandatory in international guidelines. The study showed clear parental toxicity at 150 mg/kg bw/day, accompanied with pup body weight effects. There were no specific effects on any of the reproductive parameters tested. Developmental exposure studies have found transient effects on testis morphology at 30 mg/kg bw/day, which also appears to be a threshold dose for systemic toxicity. The significance of these testicular effects is questionable, because of difficulties to repeat the findings, because of the absence of such effects in the 2-generation study, and because of general toxicity occurring at similar doses. Therefore, endocrine mechanisms most likely are not crucial for the toxicity of 4-*tert*-octylphenol.

Environment:

The report acknowledges the fact that with the exception of the extensive available information for aquatic organisms, the data set for the environment is very limited as reflected by the absence of data for terrestrial invertebrates, algae and aerial organisms.

Acute toxicity values for aquatic invertebrates and fish are in the range of 0.0133 to 0.42 mg/l and 0.077 to 3.9 mg/l, respectively. The lowest reported NOEC value for invertebrates (*Daphnia magna*) is 0.037 mg/l. For fish the NOEC values varies between 0.001 and 0.012 mg/l, with growth and mortality used as criterion for chronic toxicity in most cases. These thresholds are similar to those above in which reproduction and development are affected in aquatic organisms. Based on *in vitro* studies, these effects may be mediated by oestrogenic activity of 4-*tert*-octylphenol.

Despite the limited information regarding the concentrations of 4-*tert*-octylphenol in European surface waters, the available data suggests potential endocrine disrupting effects to aquatic organisms, particularly nearby discharge points.

Conclusion:

The CSTEE agrees with the report that 4-*tert*-octylphenol does not appear to cause critical endocrine disrupter effects in humans. The CSTEE also agrees i) that there is a potential risk to aquatic organisms nearby discharge points, and ii) that there are considerable limitations in the database**.**

2,2',4,4'-Tetrabrominated diphenyl ether

The CSTEE noted that on the basis that control of tetraBDE will result from the control of pentaBDE (particularly given that tetraBDE is not produced commercially) it has not been considered appropriate to review this substance in further detail.

Oestrone

Environment:

The authors have prepared an impressive and comprehensive review of the available literature. However, only very limited notice is given to *in vitro* studies. Recent literature on *in vitro* studies that could be helpful is provided (Murk et al., 2002; Legler et al., 2002).

Specific comments:

13.2: Missing in this section are the prime production sources: humans and livestock. The human and livestock production of oestrone is far much greater then the industrial production (estimate of natural production versus industrial should be included).

13.3.1: It should be noted that the conjugated oestrone can be rapidly deconjugated into the original oestrone by bacterial activity such as present in sewage water.

13.4.2.1: Relative potency of oestrone compared to 17-β oestradiol is not quantified. This is approximately 100. See also general comment

Missing in 13.7 is a paragraph on the likelihood that excreted oestrone enters the aquatic environment, i.e.: 1) what happens to oestrone from human faeces and urine in the STP and 2) a discussion on the likelihood that excreted œstrone enters the aquatic environment. Especially the latter is not an absolute certainty, as livestock manure is brought into the soil rather then discharged at surface water.

Missing in this section are levels in untreated waste water, which are generally the same as they only depend on the average water discharge per person, and degradation percentage in the STWs.

13.7.3.1: The focus of this section is on the production of oestrone by humans and livestock. Production, however, should be included under 13.2.

Table 13.4:

- Dutch data should include 5 STW final effluents, <0.4 - 47 ng/l, Belfroid et al 1999. (data of Johnson study refer to the same data set!) Also, the text in the Italian and Dutch sections should be corrected accordingly.
- Johnson studied 5 Italian effluents instead of 3, as mentioned in table.
- For some studies, the number of sampling locations is not mentioned.

13.7.3.1: Effluents - Netherlands

The value 12 ng/l is not mentioned in the paper by Belfroid. Nor have any measurements been done in receiving domestic waste. Also, note that measurements have been done on two occasions.

Table 13.5:

- The number of locations should be mentioned for all studies. The limit of detection (LOD) mentioned for the Dutch study is wrong (see paper by Belfroid). The LOD mentioned for RIWA is 0.3 ng. Missing is Wenzel et al 1998 (German surface water).

Conclusion:

The CSTEE agrees with the report that oestrone causes critical endocrine disrupter effects in the environment.

17b-Oestradiol

Environment:

The authors have prepared an impressive and comprehensive review of the available literature.

Specific comments:

Table 14.5:

- Netherlands: Data sets of Belfroid and of Johnson overlaps. Belfroid is original paper. Range is from <0.4 – 12.

Table 14.6:

- In this table LOD should be mentioned for Stumpf and Wegener study
- The LOD for Belfroid study is <0.3. This is depicted wrongly in text and in table.
- Missing are data of Wenzel et al 1998: Forschungsbericht 216 02 011/11. Fraunhofer-Institut, Germany.

Conclusion:

The CSTEE agrees with the report that 17β-oestradiol causes critical endocrine disrupter effects in the environment.

Ethinyl oestradiol

Environment:

The authors have prepared an impressive and comprehensive review of the available literature. However, only very limited notice is given to *in vitro* studies. Recent literature on *in vitro* studies that could be helpful is provided (Murk et al., 2002; Legler et al., 2002.

Conclusion:

The CSTEE agrees with the report that ethinyl oestradiol causes critical endocrine disrupter effects in the environment.

Conclusions:

WRC REPORT:

- As noted in the report, there is very limited knowledge of invertebrate endocrine system and ecotoxicological methodology for reproductive toxicity testing. However, in situations were there is information available on invertebrates, the report could have made more use of such data. The CSTEE also points to the almost total lack of data on potential endocrine disrupter effects in amphibians.

- According to the report, relevant grey literature for exposure and effects data was obtained by consultation with the relevant sector groups of CEFIC responsible for the different chemicals and by contacting the European Environment Agency for environmental monitoring data (page 2-4). However, the availability of industry-proprietary data for the assessment is not clear.

- The CSTEE noted that recent literature data were not included, but was informed that the cut-off date for literature searching for effects and exposure data was March/April 2002. For some chemicals, the CSTEE has referred to relevant literature published after the cut-off date.

- CSTEE noted that for compounds with an identical mode of action, such as oestrogenic hormones and xeno-oestrogens that act through an oestrogen receptor, the performance of individual risk assessments is problematic. For example, the effects of natural and synthetic oestrogens may be additive, especially since these compounds often co-occur in the aquatic environment.

- CSTEE further noted that a problem encountered in the assessment and interpretation of the data concerns the low and variable detection levels of various compounds, in particular the oestrogenic hormones: the detection limits for these compounds were in the range of, or above concentrations at which (oestrogenic) effects have been shown on fish.
- The CSTEE agrees with the overall conclusions for the three oestrogens, but notes that the report sometimes contains errors and that limited notice is given to *in vitro* studies.

BKH REPORT:

- There is general agreement that the approach taken in the report has greatly improved compared to the 2000 report. In particular, exposure and persistency data are now adequately used. Missing data on environment is clearly identified in the report.

- The CSTEE agrees with the report that reproduction toxicity was classified as systemic toxicity and not as endocrine disruption, unless specific parameters were affected such as hormone levels.

- Low production volume chemicals but with high release in the environment or with high potency are not sufficiently covered in the report.

- Comments on wildlife are generally good, but it must be specified that potential endocrine disrupter effects are different for the health of human individuals as compared to wildlife populations.

- The use of terms such as *classification* and *labelling* may give rise to considerable misunderstanding in view of their current use in the European Union.

- Type of release is not taken into account in the report: industrial chemicals often result in continuous release, pesticide use often results in temporal release.

- It is not valid and even misleading to relate the endocrine disrupter effect of non-oestrogenic compounds with the endocrine disrupter effect induced by the natural ligand 17β-oestradiol *in vivo* in rats (4.2, pages 17 and 18; 6.1, page 34).

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