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

Opinion

on the results of the Risk Assessment Report of:

DIBUTYLPHTHALATE

CAS No.: 84-74-2
EINECS No.: 201-557-4

Carried out in the framework of Council Regulation (EEC) 793/93 on the evaluation and control of the risks of existing substances[1]

expressed at the 23rd CSTEE plenary meeting

Brussels, 24 April 2001

[1] Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of those substances if they are produced or imported into the Community in volumes above 10 tonnes per year. The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94, which is supported by a technical guidance document.

Terms of reference

In the context of Regulation 793/93 (Existing Substances Regulation), and on the basis of the examination of the Risk Assessment Report the CSTEE is invited to examine the following issues:

1. Does the CSTEE agree with the conclusions of the Risk Assessment Report?

2. If the CSTEE disagrees with such conclusions, the CSTEE is invited to elaborate on the reasons for this divergence of opinion.

Introduction

Dibutylphthalate (DBP) is in general mainly used as a plasticiser in resins and polymers such as polyvinyl chloride. DBP is also used in printing inks, adhesives, sealants/grouting agents, nitrocellulose paints, film coatings and glass-fibre products. The substance is also used in cosmetics as a perfume solvent and fixative, a suspension agent for solid aerosols, a lubricant for aerosol valves, an antifoaming agent, a skin emollient and as a plasticiser in nail polish and fingernail elongators. Consumers may be exposed to DBP via cosmetics (nail polish and enamels), adhesives and regenerated cellulose film-wrapped food and children's toys. In general consumer exposure is low. Indirect exposure via the environment may occur from DBP released through wastewater effluents and air at the sites where it is produced, formulated and/or processed/used. Local air concentrations are low (μg/m^3 range). Also the total daily intake is low (μg/kg bw/day).

GENERAL COMMENTS

The environmental and health parts of the document are both of high scientific quality. The conclusion i) requiring more information for the environmental risk assessment is supported by the CSTEE. The conclusion is not only justified by a risk for the atmospheric compartment, but also by the need to better assess local PEC in surface waters and by the need for information on endocrine disrupting effects on aquatic organisms. The CSTEE agrees with the general conclusion ii) for the consumer and man indirectly exposed via the environment. The CSTEE concludes that for all workplace exposure scenarios conclusion ii) is appropriate for the risk of systemic effects of repeated-dose exposure and for reproductive effects of DBP. Thus, the proposal of the *rapporteur* of conclusion iii) for systemic effects from dermal exposure due to aerosol forming activities is not supported. The CSTEE agrees with the general conclusion that there is a need to limit the risk of local effects in the respiratory system following repeated exposure in the workplace.

The CSTEE is aware of new studies concerning sensitisation, mutagenicity and developmental toxicity. A short summary of these studies is given in the enclosed Annex. However, it is the opinion of the CSTEE that these studies do not change the overall conclusions.

The CSTEE supports the use of assessment factors when they are based on solid scientific data. However, the CSTEE does not generally support the use of default assessment factors beyond those used for interspecies and intraspecies uncertainty and variability.

The CSTEE encourages the use of original references whenever possible. Key studies used to derive NOAEL/NOAEC values, should be described in sufficient detail to allow the reader to judge the quality of the study and if stated alterations in effect parameters are of sufficient magnitude to consider the effects as adverse.

SPECIFIC COMMENTS

Environment

Exposure assessment

There are some controversial aspects in the exposure assessment for the aquatic environment at the local scale.

Regional PECs are properly calculated according to TGD and are in reasonable agreement with available monitoring data. Local PEC, deriving from production and processing activities seem quite low in comparison with regional figures (less than one order of magnitude higher) and some measured figures are substantially higher. In some cases, information to calculate local exposure is not reported for confidentiality reasons.

The comparison between calculated and measured levels at the local scale is not properly reported in the document.

Effects assessment

Aquatic organisms

The information available on short and long term toxicity for fish invertebrates and algae is rather comprehensive. The PNEC for aquatic organisms resulting from this information is supported by the CSTEE.

Nevertheless, DBP is known as an endocrine disrupting chemical. In the report it is mentioned that several long term tests are carried out with fish (data of only one study are reported) but it is highlighted that reproductive effects or other specific endpoints with respect to estrogenic activity were not studied in these tests.

Reproductive tests on partenogenetic invertebrates may be not suitable to assess estrogenic effects. The CSTEE is of the opinion that more information is needed on this aspect.

The PNEC for micro-organisms is properly calculated on the basis of the available information. Nevertheless the value of 0.22 mg/L is probably too conservative, taking into account that no impairment of biodegradation was observed at concentrations orders of magnitude higher.

Terrestrial organisms
The report presents a proper assessment of the available information. The PNEC for terrestrial organisms is supported by the CSTEE.

In the absence of proper guidelines in the TGD for assessing the effects on the atmospheric compartment, the report includes and ad-hoc assessment for DBP. The assessment is scientifically sound and identifies terrestrial plants as the key ecological receptor. The rationale for the derivation of PNEC for plants is clearly presented and is considered appropriate.

The report includes a proposal for classification as R54 toxic to flora. This proposal confirms, once more, the need for developing classification criteria for the terrestrial environment, previously expressed by the CSTEE

Risk characterisation

If some of the high concentrations measured at local level are used, a PEC/PNEC ratio substantially higher than 1 can be calculated. These values were not used in the report.

If there are no reasons for rejecting measured data as not reliable or representative, the highest figures should be used as a worst-case scenario for reliable risk assessment.

The conclusion requiring more information for the environmental risk assessment is supported by the CSTEE. Nevertheless, something should be added to justify the conclusion.

In particular:

More information is needed to assess local PEC in surface water and to better evaluate the disagreement between calculated and measured figures.

More information is needed on endocrine disrupting effects on aquatic organisms.

Human Health

Exposure assessment

Occupational

DBP is primarily (76%) used as a plasticiser in polymers (mainly PVC, but also rubber). 14% are used in adhesives, 7% in printing inks, and the remaining 3% in miscellaneous other preparations. In 1998 it is estimated that 26,000 tonnes were produced in the EU, of which 8,000 tonnes were exported outside the EU. There appears to be a clear decreasing trend in the production of DBP. Occupational exposure occurs both via the inhalation and dermal routes. The highest exposure levels in air during DBP production (up to 5 mg/m^3, most likely 2 mg/m^3) occurs during sampling and filling. Short-term exposures may be in the range of 10 mg/m^3. The worst-case scenario with respect to dermal exposure during production of DBP is estimated (EUSES) to be approximately 400 mg/day. The actual dermal exposure levels are likely to be substantially less. Similar exposure levels can be reached during polymer processing. Air concentrations of DBP during use of DBP-containing products causing aerosol formation, could be as high as 10 mg/m^3, most likely 2 mg/m^3. During non-aerosol forming activities inhalation exposure levels are negligible.

Consumers

DBP-containing products are used in various consumer situations. The sum of adult exposures is expected to be less than 0.35 mg/kg for acute exposure and 0.03 mg/kg/day for repeated exposure. For infants exposed via toys the exposure is considered to be < 1 μg/kg/day.

In a recent study by Blount et al. (2000), urinary levels of MBP were measured in a reference population. The levels found are in accordance with the exposure estimates mentioned above. However, the measurements point to women of reproductive age as a potential high-exposure group. This finding is important because *in utero*/perinatal exposure seems to be the most critical exposure period for DBP toxicity.

Indirect

The indirect exposure of man via the environment through air and drinking water has been estimated using EUSES at the local scale and depends on production, processing, and formulation. The highest daily intake, approximately 0.1 mg/kg/day, seems to be related to the processing of polymers.

Effects assessment

DBP is rapidly absorbed and excreted after oral exposure. In rats dermal absorption appears to be in the range of 10% of the oral absorption. From an *in vitro* study it seems that human dermal absorption is only 2.5% of that in rat. It is the opinion of the CSTEE that this is not sufficiently discussed in the risk assessment. The CSTEE emphasises the need for more data to quantitate the probable difference in dermal absorption between humans and experimental animals. No data on absorption following inhalation exposure are available. The major part of absorbed DBP is hydrolysed to the monoester metabolite and further glucuronidated, or is subjected to oxidation leading to hydroxy and/or keto metabolites. According to a study by Coldham et al. (1998, not cited in the RAR) the monoethyl phthalate metabolite can also be formed (at least in cattle). MBP and DBP are able to cross the placenta. No clear accumulation of DBP or MBP in specific tissues or organs were detected.

It is generally assumed that free MBP is the active, toxic metabolite of DBP. For the evaluation of the sensitivity of humans to DBP toxicity it is important to have information on the level of free MBP in the target tissues in humans compared to that in test animals. In the study by Blount et al. (2000), it was found that human urinary MBP was predominately conjugated as the glucuronide form. However, in 5% of the tested urinary samples the authors found a substantially higher concentration of unconjugated MBP.

The CSTEE agrees that the acute toxicity of DBP is low, but emphasises that the acute toxicity following inhalation exposure is difficult to assess. The CSTEE agrees that DBP is not a skin or eye irritant. However, irritation of nasal mucous membranes have been reported for mice after exposure by inhalation for 2 h to 0.25 mg/L and cats after receiving 1 mg/L for 5.5 h. Repeated exposure of rats by inhalation to concentrations ≥ 1.18 mg/m^3 induced adverse histopathological effects in the nasal cavity and larynx. These local, irritating effects in the upper respiratory tract give cause for some concern, however, due to an obvious lack of inflammation the CSTEE agrees with the conclusion of the RAR that DBP should not be classified as a respiratory irritant. The rationale for not including the Voronin (1975) study should be given in section 4.1.2.3.3.

DBP has not been shown to be a skin sensitiser in well-accepted animal tests. Allergic dermatitis in humans has been reported in several studies using antiperspirants, nail polish and after contact with plastics containing DBP (watchbands, etc). A new case-study by Gall (1999) also reports an incident of anaphylactic shock in a 21-year old patient after taking one Gelomyrtol forte capsule also containing 7.5 mg DBP. Prick tests were carried out with all the individual substances from Gelomyrtol forte capsules, and the only positive test came from DBP, giving rise to a weal of 3 cm, which expanded to cover the entire lower arm. Although this single study does not have implications for the conclusions on the sensitising properties of DBP, the CSTEE suggests that it should be included in the RAR.

The CSTEE is aware of studies that indicate that dermal exposure to DBP may enhance the sensitisation potential of other skin sensitisers, possibly by acting as an adjuvant. A study conducted in 1996 by Dearman et al., reports on an adjuvant effect of DBP on dermal sensitisation in mice exposed to fluorescein isothiocyanate (FITC), a skin sensitising flurochrome. DBP, in a dose-dependent fashion augmented the ability of topically applied FITC to stimulate proliferative responses in mice by draining lymph node cells, a correlate of skin sensitising potential. DBP also increased the frequency of lymph node dendritic cells bearing antigen (FITC positive DC), and increased the median amount of FITC antigen per dendritic cell. *In vitro* skin absorption studies also indicated that DBP increased the dermal absorption of FITC marginally. Exposure of mice to DBP alone did not give rise to any of the mentioned effects. The CSTEE finds that the possible adjuvant effects of DBP on other skin sensitisers should be commented upon in the RAR, and more studies on the adjuvant effects of DBP both on skin and respiratory sensitisers are warranted.

Several repeated-dose oral studies have been conducted in mice and rats. The quality of the reported studies varies, and some are not suitable for risk assessment. The key studies appear to be the NTP (1995) mice and rat studies and the rat study by Schilling et al. (1992). The NOAEL of 152 mg/kg/day from the Schilling study has been used in the risk assessment. In these studies changes in several haematological parameters have been used as the critical effect used to determine the NOAEL. The Schilling study is not available in the open literature (confidential study by BAYER). A more detailed description of this study in the

RAR is needed in order to assess the quality of this study. It is not clear how many doses were used, from the text it could be only two doses. The NTP studies are well performed. The CSTEE recommends that the NOAEL of 177 mg/kg from the NTP rat study (based on statistical significantly decreased haemoglobin values and erythrocyte counts together with increased numbers of blood platelets) should be used as basis for the risk assessment of systemic effects. The NTP studies, as well as several other oral studies of varying lengths, clearly show that the testis is a target tissue for DBP toxicity. In animals there are clear species differences to DBP-induced testicular toxicity. The CSTEE agrees with the RAR that the NOAEL for peroxisome proliferation is not used in the risk assessment.

There is no adequate dermal repeated-dose toxicity study. The CSTEE supports the identification of the local LOAEC of 1.18 mg/m^3 (epithelium changes of nasal cavity) derived from the 28-day inhalation study by Gamer et al. (1999). No systemic toxic effects were noted at the highest concentration tested (509 mg/m^3). The limited scientific value of this study for risk assessment purposes due to what appears to be too low exposure concentrations, should be discussed. According to OECD TG 412 the highest concentration should give signs of systemic toxicity. The highest concentration in the Gamer study is estimated to be in the same range as the NOAEL for the repeated oral 90 days study. It is the opinion of the CSTEE that DBP has not been tested at sufficiently high concentrations in the inhalation study to be able to detect systemic toxicity. Also, the indication that MBP is a proximate toxic metabolite of DBP and a possible first pass effect in the liver in oral versus in inhalation studies should be kept in mind. The fact that this is a 28-day study makes also it less useful in order to derive a NOAEC for risk assessment purposes. Identification of a scientifically well defined NOAEC is important since it is used to draw conclusions during risk characterisation of worker exposures, especially with respect to aerosol-forming activities. The human epidemiological studies on neurological symptoms are of limited value due to small sizes of the exposure groups, lack of appropriate controls and mixed exposures.

An overall evaluation of bacterial mutagenicity tests shows that DBP is not a bacterial mutagen. Furthermore, no cytogenetic effects have been noted in various *in vitro* cell systems. In addition, DBP was negative in one cell transformation test. The negative response in the cell transformation test has recently been confirmed (Barber et al., 2000, not in RAR). Two *in vivo* micronucleus tests are negative. Unfortunately, most of the reported *in vitro* mutagenicity studies have not been reported in sufficient detail to allow an evaluation regarding their quality. Regarding gene mutation in mammalian cells the results are somewhat contradictory. The CSTEE has evaluated a new mouse lymphoma test (Barber et al., 2000) that shows a positive effect in the presence, but not in the absence of a metabolism system (S9 mix). The fact that one study showed negative effects without S9 mix (not tested with S9 mix), but two were positive in the presence of S9 mix, indicates that DBP may cause gene mutation in cells in the presence of a metabolic activation system. However, recognising the relatively high rate of false positives in the mouse lymphoma assay and the overall negative responses in all other tests, the CSTEE agrees that DBP cannot be characterised as being genotoxic. Interestingly, a recent German study by Kleinsasser et al. (2000) indicates that DBP may cause DNA strand breaks in human mucosal cells *in vitro* derived from the oropharynx and inferior nasal turbinate. The concentration of DBP used in the study was very high (354 µmol/ml). Thus, it cannot be ruled out that the observed effect on DNA could be of secondary nature. It could be of interest to have further studies clarifying a possible genotoxic effect of DBP on human mucosal cells of the upper respiratory system.

DBP has not been tested for carcinogenicity in experimental systems, nor are there any human data available. DBP has been documented to enhance peroxisome proliferation in rats and mice. Many peroxisome proliferators have been shown to cause liver tumours when given at high doses and for long periods in mice and rats. Based on the observations that humans are non-responsive to peroxisome proliferation, the CSTEE agrees that the peroxisome proliferative effect of DBP in rats and mice is of no relevance to humans.

The male reproductive system is considered to be a main target of DBP toxicity. A recent study by Mylchreest et al. (2000) established NOAEL (50 mg/kg) and LOAEL (100 mg/kg) values for toxicity of DBP on male reproductive development in the F1 generation. The CSTEE considers this study to be very relevant for the risk assessment of reproductive toxic effects of DBP. This study should be used together with the 2-generation rat study that established a LOAEL of 52 mg/kg for embryotoxicity in the F2-generation, in the evaluation of the risk of reproductive toxicity. This point is specified in the subsequent comments to the risk characterisation.

The CSTEE would like to draw attention to 4 additional studies by Ema et al. (2000), Ashby and Lefevre (2000), Shultz et al., (2000) and Andersen et al. (1999). Short comments on the findings in these studies with direct relevance to DBP toxicity are found in the Annex. None of these four studies affect the conclusions of the risk assessment, but they address potential mechanisms of DBP toxicity and periods of susceptibility.

Risk characterisation

Workers

The CSTEE agrees with conclusion ii) with respect to acute toxicity, skin and eye irritation, corrosion, mutagenicity and carcinogenicity. Although there are not sufficient data to classify DBP with R37, there is some concern about respiratory irritation of the upper airways based on the effects reported in acute inhalation studies in mice and cats. However, these concerns are not sufficient to warrant conclusion iii) in agreement with the RAR.

In the evaluation of the risk from dermal exposure, the RAR arrives at conclusion iii) for the aerosol-forming activities scenario, and conclusion ii) for the other scenarios. The CSTEE recommends that the NOAEL from the oral study, and not the NOAEC from the inhalation study, be used for extrapolation to the dermal exposure situation (NOAEL 177 mg/kg from the NTP study). Conclusion ii) is thereby warranted for all dermal exposure scenarios with respect to repeated dose toxicity. When calculating the MOS values for repeated-dose inhalation toxicity (systemic effects), it appears that the atmospheric concentrations and not the inhaled dose is used. A NOAEL in mg/kg bw/day should be calculated from the NOAEC of 509 mg/m^3 in the 28-day rat study. Similarly, the anticipated human dose should be calculated based on the air concentration of DBP in the working atmosphere, inhalation volume during work-hours and body weight.

The CSTEE agrees with conclusion iii) with respect to local effects following repeated inhalation exposure for exposure scenarios 1, 2 and 3a. The CSTEE also agrees with conclusion ii) with respect to systemic toxicity from repeated inhalation exposure.

Risk of reproductive toxicity after dermal and inhalation exposure

The CSTEE agrees with conclusion ii) with respect to reproductive toxicity following all dermal and inhalation exposure scenarios. However, the MOS/risk-ratio values for both the dermal and the inhalation exposure scenario 3a (aerosol-forming activities) are borderline between conclusions ii) and iii). Conclusion ii) is supported also for the aerosol forming activities scenario for the two following reasons:

1) A LOAEL value of 52 mg/kg has been established based on embryotoxicity in the F2 generation (decreased survival and decreased body weight of the offspring). The NOAEL value of 50 mg/kg established for reproductive effects in the F1 generation by Mylchreest et al. (2000) supports the choice of a low uncertainty factor (2x) when extrapolating from the LOAEL value to a NOAEL value.

2) The internal exposure for DBP/MBP is most likely overestimated by assuming 10% and 100% absorption from dermal and inhalation exposure, respectively. *In vitro* data from dermal absorption studies in rats and humans indicate that the rate of dermal absorption in humans is substantially less than in rats (approximately 2.5% of that in rats). This strengthens conclusion ii) with respect to dermal exposure.

Consumers

The CSTEE agrees with the general conclusion ii) of the RAR for consumer exposures for all effect types.

The exposure scenario II for consumers refers to short-term inhalation exposure (4 hours, once a year) after the use of DBP in adhesives, e.g. used for glueing carpets. The risk characterisation is based on acute exposure and acute toxicity. However, the CSTEE wonders whether long-term exposure to DBP also occur indoors from similar uses. If that is the case, the risk characterisation should be based on long-term as well as short-term exposure.

Indirect exposure

The CSTEE agrees to the conclusion ii) of the RAR for consumer exposures for all effect types.

Annex

HR Andersen et al.: ***Comparison of short-term estrogenicity tests for identification of hormone-disrupting chemicals*****. Environ Hlth Persp 107 (Suppl. 1), 89-108, 1999.**
In this study several tests for detecting oestrogenicity of chemicals were compared. One of the conclusions from this study is that DBP is a weakly oestrogenic compound that is inactive in most assays, but active in the MCF-7 cells and yeast protocols.

BC Blount et al.: ***Levels of seven urinary phthalate metabolites in a human reference population*****. Environ Hlth Perspect 108, 979-982, 2000.**
The authors have measured urinary levels of monoester metabolites of several phthalates, including DBP, in a limited human reference population (289 subjects). The urinary MBP levels were: 95th percentile, 294 ppb or 162 µg/g creatinine. This value equals an intake of DBP of approximately 7 µg/kg/day (calculations made in the Correspondence part of the EHP journal). Such an intake level is in agreement with the MAFF intake estimates cited in the RAR. However, women of reproductive age were found to have significantly higher levels of MBP than other age groups. The 95th percentile of this group had an estimated daily intake of DBP of 32 µg/kg, whereas the maximum urinary MBP levels in the reference population equals a daily intake of approximately 115 µg/kg. These values do not change the conclusions of the RAR. However, the values point to women of reproductive age as a potential high-exposure group. This finding is important because *in utero*/perinatal exposure seems to be the most critical exposure situation for DBP toxicity.

E Mylchreest et al.: ***Dose-dependent alterations in androgen-regulated male reproductive development in rats exposed to di(n-butyl)phthalate during late gestation.*** **Tox Sci 55, 143-151, 2000.**
This study describes the effects of a 10-day prenatal exposure (day 12-21) to DBP at doses from 0.5 mg/kg/day to 500 mg/kg/day. The authors report a NOAEL of 50 mg/kg/day and a LOAEL of 100 mg/kg/day based on the presence of retained areolas or nipples in exposed male offspring. This is the lowest NOAEL described for developmental effects on the F1 generation.

M Ema et al.: ***Critical period for adverse effects on development of reproductive system in male offspring of rats given di-n-butyl phthalate during late pregnancy.*** **Toxicol Lett 111, 271-278, 2000.**
Pregnant rats were exposed to DBP (doses ranging from 500-1500 mg/kg/day) in 3-day periods to determine the period of highest susceptibility for toxicity to the developing male reproductive system. The authors found that day 15-17 of pregnancy was the most susceptible for DBP-induced undescended testes and decreased anogenital distance.

J Ashby and PA Lefevre: ***The peripubertal male rat assay as an alternative to the Hershberger castrated male rat assay for the detection of anti-androgens, oestrogens and metabolic modulators.*** **J Appl Toxicol 20, 35-47, 2000.**

This study was performed to compare the peripubertal male rat assay with other assays for the detection of endocrine disrupters and metabolic modulators. DBP was one of the tested substances. Exposure to DBP (500 mg/kg/day) in the period immediately following weaning (post natal days 22-36) resulted in decreased reproductive tissue weights, whereas exposure during day 36-50 did not have an effect.

From this study it appears that DBP differs from several classical anti-androgens by requiring exposure in a restricted post-weaning period for effects on reproductive tissue weights to occur.

VD Shultz et al.: ***Microarray analysis of altered gene expression in the testes of foetal rats exposed to DBP.*** **Abstract, Teratology, 61 455, 2000.**

Microarray analysis of altered gene expression was performed in the testes of foetal rats exposed to DBP (500 mg/kg/day) from gestation days 12 to 21. The mRNA level of P450-side chain cleavage, which is a rate limiting step in testosterone biosynthesis was altered by DBP exposure. In addition, the mRNA levels of testosterone repressed prostate message-2 (TRPM-2) was induced. The authors suggest that DBP toxicity may be mediated by an inhibition of testosterone biosynthesis. This study is too limited to establish a link between DBP and testosterone levels. However it is relevant for the discussion of the seemingly anti-androgenic effects of DBP.