RIVM report 601900004 / 2003

The relevance of developmental toxicity endpoints for acute limit setting

M.T.M. van Raaij, P.A.H. Janssen, A.H. Piersma

This investigation has been performed by order and for the account of The Ministry of Public Health, Sports and Well-being, within the framework of project 601900, Acute toxicity and risk assessment.

RIVM, P.O. Box 1, 3720 BA Bilthoven, telephone: 31 - 30 - 274 91 11; telefax: 31 - 30 - 274 29 71

Samenvatting

Toxicologische limieten op basis van gezondheidseffecten voor de mens staan in de belangstelling (bijvoorbeeld ARfDs en AEGLs). Ontwikkelingstoxiciteit wordt in het algemeen beschouwd als een relevant eindpunt voor het vaststellen van acute limieten. Dit soort toxiciteit wordt normaal onderzocht in dierstudies die worden uitgevoerd volgens vaststaande richtlijnen waarbij dieren gedurende 10-14 dagen tijdens de organogenese worden blootgesteld. Echter, het is de vraag of de NOAELs die in deze studies worden vastgesteld altijd representatief zijn voor een éénmalige blootstelling. In dit rapport worden NOAELs en LOAELs van éénmalige blootstellingen vergeleken met NOAELs en LOAELs van studies met herhaalde blootstelling gebaseerd op de richtlijnen. Dit werd gedaan voor diverse eindpunten afzonderlijk (maternale toxiciteit, resorpties, foetaal lichaamsgewicht, aantal foetussen met malformaties, effecten op het skelet en specifieke malformaties). Indien er geen of weinig verschil wordt gevonden tussen NOAELs/LOAELs van éénmalige en herhaalde blootstellingstudies wordt het eindpunt (de NOAEL) uit de herhaalde blootstellingstudie beschouwd als representatief voor een éénmalige blootstelling. Indien er grote verschillen worden gevonden is de NOAEL voor een bepaald eindpunt uit de herhaalde blootstellingstudie niet relevant.
Op basis van deze analyse wordt geconlcudeerd dat maternale toxiciteit (lichaamsgewicht, voedselopname, orgaangewichten, klinische verschijnselen) geen geschikt eindpunt is voor het vaststellen van limieten voor acute blootstelling. De relevantie van foetal lichaamsgewicht (en vertraagde ossificatie) voor het vaststellen van acute limieten moet worden geëvalueerd binnen de totale context van effecten op de foetus en de moeder. Resorpties worden beschouwd als relevante eindpunten voor acute limieten. Malformaties en effecten op het skelet worden beschouwd als relevante eindpunten tenzij er informatie beschikbaar is die anders uitwijst. Het gebruiken van een NOAEL uit een ontwikkelings-studie volgens de gangbare richtlijnen (herhaalde blootstelling) verschaft te allen tijde een conservatieve (veilige) inschatting van de NOAEL bij een éénmalige blootstelling.

Summary

Health-based limits for acute exposure of humans have received increasing attention (e.g. ARfDs and AEGLs). Developmental toxicity is in general considered to be a relevant endpoint for setting such limits. Developmental toxicity is normally investigated in 'guideline-based' animal studies which involve repeated dosing for 10-14 days during organogenesis. However, it can be questioned whehter NOAELs observed in such developmental toxicity are representative for single exposure situation. In this report, the NOAELs and LOAELs of single dose studies were compared to the NOAELs and LOAELs in normal 'guideline-based' repeated dose studies for several effects separately (maternal toxicity, resorptions, fetal body weight, number of fetuses with malformations, skeletal effects, and specific malformations). When no or limited differences are observed between single and repeated NOAELs/LOAELs, the NOAEL of a specific endpoint observed in a repeated dose study is relevant for setting an acute limit. When large differences occur, NOAELs for that endpoint are not an appropriate starting point for setting an acute limit. Based on this analysis it was concluded that gross maternal toxicity (maternal body weight, food intake, organ weights, clinical signs) is not an appropriate starting point for setting limits for acute exposure. The relevance of fetal body weight (and retarded ossification) for acute limit setting should be evaluated within the total context of developmental effects and maternal toxicity. Resorptions are considered relevant for setting acute exposure limits. Malformations and skeletal effects are considered relevant starting points for acute limit setting unless evidence is available to indicate otherwise. Using a NOAEL from a normal 'guideline-based' repeated dose developmental toxicity study always provides a worst case estimation of the NOAEL in a single dose exposure.

Contents

1. Introduction and Objectives

Acute exposure in risk assessment

Acute exposure to toxic substances and the risk assessment of such exposures have received increasing attention in the last decade. This is – in part – due to a number of chemical incidents in the Netherlands. Apart from this, acute exposure has become an important issue also in some regulatory frameworks. Examples are: setting of the Acute Reference Dose (ARfD) for pesticides, setting of Acute Exposure Guideline Levels (AEGLs) or similar emergency response values, and risk assessment of biocidal products that are used only once or in low frequency (within the scope of the Dutch Pesticide Act). It has become clear that for several aspects of the acute risk assessment toxicological knowledge is limited. In the project 'Acute toxicity and risk assessment' some of the specific gaps of knowledge are investigated in order to provide some improvement of the toxicological risk assessment process. One of those gaps is the use of developmental toxicity endpoints for acute limit setting.

Acute health-based limit values are set to protect the public from adverse health effects of (very) short periods of exposure. The ARfD is an oral limit set for 24 hours or less. Emergency response guideline values such as the AEGLs are set for periods of 10 minutes up to 8 hours. For the setting of such acute exposure limits, toxicological endpoints are selected that may be relevant for such an acute exposure. For example, liver hyperplasia that is observed only in semi-chronic or chronic toxicity studies is not a relevant endpoint for acute limit setting. On the other hand, acetyl cholinesterase inhibition by pesticides can occur already after a single dose and is therefore a highly relevant endpoint for setting of an ARfD. A detailed discussion on the selection of endpoints for setting of the ARfD and AEGLs is provided by Van Raaij (2001) and NRC (2001).

Acute exposure and developmental toxicity

One type of endpoints that is considered to be relevant for acute limit setting is developmental toxicity. This is because it has been shown that teratogenic effects (malformations) can occur after a single exposure when the substance is given in the critical time window during pregnancy. Starting from this perspective, many toxicologists and organizations do consider all effects seen in regular guideline-based developmental toxicity studies as relevant for acute limit setting (Dewhurst, 2000; Billington and Carmichael, 2000). The standard procedure uses the NOAEL from a developmental study (regardless of the endpoint on which the NOAEL is based) to set an acute limit by using assessment factors. It can be questioned whether this approach is appropriate. In a regular developmental study, i.e. basically according to OECD guideline 414, the animals are exposed repeatedly for a number of days during organogenesis. Mice and rats are exposed from gestation day (GD) 6-15 (total 10 days), and rabbits are exposed from GD 6-18 (total 13 days) but exposure may be also continued to one day before birth. This period covers about 50% of the gestation period in animals. Compared to other studies in a toxicological database, this exposure period is rather short. However, compared to a defined acute exposure of humans (AEGLs range from 10 min

to 8h; ARfD is for < 24h), this type of exposure resembles more a repeated dosing regime. A one day exposure of a pregnant woman covers less than 1% of the total gestation period. The observed effects at the end of the gestation period (at about GD 20) in an animal study may actually reflect repeated dose toxicity rather than acute toxicity effects. It could be argued that the NOAELs of various effects (=endpoints) observed in regular developmental toxicity studies are highly variable with respect to their relevance as a starting point for acute limit setting. In this respect, a NOAEL that is based on teratogenic effects could be considered relevant for acute limit setting because of the critical window concept. In contrast, more or less general fetotoxic endpoints such as fetal body weight reduction or delayed skeletal ossification may in many cases be considered less relevant for acute exposure since they may be the consequence of repeated dose exposure. Hence, the NOAEL for such endpoints is not considered to be an appropriate starting point for acute limit setting since the NOAEL for such an effect is probably much higher in a single day experiment.

In a situation where an acute limit is derived from a study using a longer exposure duration, the acute limit may be set too low, i.e. too conservative. This may have undesirable consequences. In case of a too low ARfD some pesticide may be denied access on the market or actual risk assessment will be hampered in case of residues that exceed the limits. In case of AEGLs that are too conservative, unnecessary emergency response actions will take place which may in fact provide more hazard to the public (e.g. in case of an evacuation) than the chemical hazard itself. Therefore, it is important that acute limits are set on toxicological endpoints that are relevant for acute exposure. Although most ARfD's have been set using a total assessment factor of 100 also smaller factors may be used depending on the data available. Emergency response guidelines values are mostly set using smaller assessment factors (3 – 30). Therefore, a difference between the $NOAEL_{single}$ and the $NOAEL_{rep}$ of about 3-fold may already have serious consequences for setting an acute limit.

At present for about 15-30% of the substances, the ARfD's set by different organizations are based on developmental or reproduction toxicity endpoints. In addition, the AGW* for about 10% of hazardous substances with Emergency Response Intervention Values in the Netherlands (Ruijten et al., 2000). Therefore, developmental toxicity represents an important endpoint for acute limit setting for a substantial number of substances.

In addition to the issues of acute limit setting, also actual risk assessment of developmentally toxic substances could be improved in cases of exceeding limits if one could differentiate between effects on the basis of their degree of relevance for acute exposure.

Objectives of the study

In this study we determine which type of developmental toxicity endpoints (and associated NOAELs) observed in standard guideline-based studies are relevant or less relevant for acute limit setting. However, the issue is complex because both dosing length (number of gestation days) as well as dose level play a role. In below, the solid line represent the opening and

* AGW = Alarmerings GrensWaarde: similar to AEGL-2 or ERPG-2 levels.

closing a critical time window for a specific developmental effect. The window begins on day 6, is maximal at day 8 and is closed on day 11. A low dose (broken line) will be able to affect the fetus only during a small period of time when the sensitivity is maximal. However, a higher dose (dotted line) is able to affect the fetus over a longer period of time.

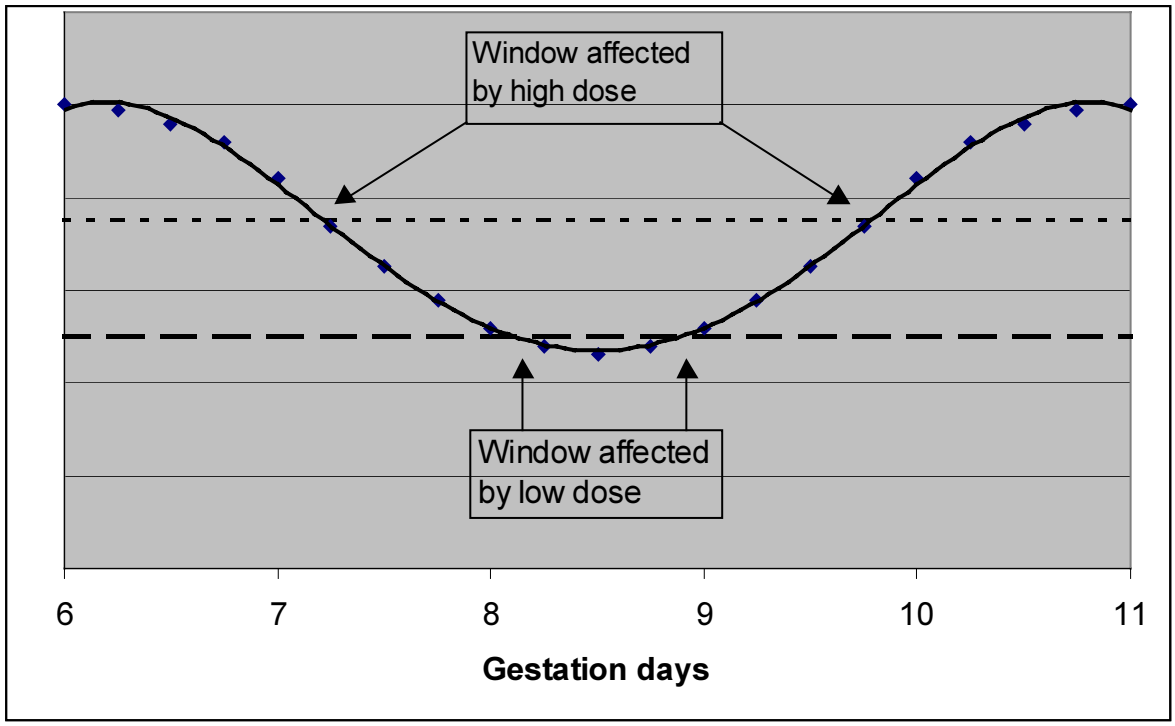

Figure 1. Schematic representation of the window of sensitivity over time (solid line), and the consequence of a single low dose (dashed line) and a single high dose (dotted line).

Taken together, when comparing NOAELs/LOAELs from single dose studies with NOAELs/LOAELs from repeated dose studies, it is expected that the difference between such values is indicative for the likelihood that such an effect may or may not occur during a single exposure. Therefore, we chose to perform this by a NOAEL and LOAEL ratio comparison between single dose and regular guideline-based developmental toxicity studies. For effects that are relevant for a single exposure, it is expected that the NOAEL/LOAEL values should be about the same in single dose and repeated dose studies respectively. For such effects it is appropriate to use the NOAEL from a regular developmental toxicity study to set an acute limit value. However, for effects that are caused by repeated dosing rather than by a single dose, it is expected that the NOAEL/LOAEL in a single dose study are higher than those in a regular (repeated dose) developmental toxicity study.
In this report we have focussed on the following effects: maternal toxicity, embryonal/fetal resorptions, fetal body weight, percentage of abnormal fetuses, skeletal variations (delayed ossification), and some specific malformations and variants.

2. Methods

In this investigation NOAELs and LOAELs for specific effects in single dose developmental toxicity studies are compared with the NOAELs and LOAELs for the same effects in a regular – repeated dose – developmental toxicity study. In order to perform such an investigation one should first identify substances for which this type of information is available. The availability of single dose studies was considered to be pivotal since it was expected that regular repeated dose developmental toxicity studies should be easier to find. For the identification of substances for which single dose developmental toxicity studies would be available, two procedures were used. First, experts and colleagues within our institute (RIVM) were requested to propose candidate substances for this analysis. Second, a literature search was performed using a range of keywords and combinations. The searches were performed in TOXLINE 1985-2001 and MEDLINE R+. In addition, for several substances additional information was found using the Developmental and Reproductive Toxicology/Environmental Teratology Information Center (DART®/ETIC) Database (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?DARTETIC).

For those substances for which single dose studies were identified, an additional search was performed to obtain repeated dose developmental toxicity studies. In addition, monographs (e.g. ATSDR profiles, Environmental Health Criteria, WHO-JMPR) and databases (e.g. IUCLID database) were used to obtain repeated dose (guideline-based) developmental toxicity studies. Repeated dose studies should have a dosing schedule of ≥ 7 days during gestation, preferably according to OECD 414. However, sometimes longer exposures were used (including some exposure before copulation).The repeated-dose studies should be performed with the same species, the same chemical /derivate (e.g. cadmium chloride is compared to cadmium chloride but not to cadmium acetate), the same route of exposure, and in case of gavage dosing similar vehicles.

The selected studies were then evaluated for a range of developmental endpoints as given in the tables in Appendix B. For each effect, the NOAEL and LOAEL in the study was determined. Finally, for each effect separately, the NOAEL from the single dose study ($NOAEL_{single}$) was compared to the NOAEL from the repeated dose study ($NOAEL_{rep}$). When more than one study was available the lowest LOAEL was used in combination with the highest NOAEL that was below the lowest LOAEL. In case some NOAELs may be above LOAELs in other studies, expert judgement was used to select the NOAEL and LOAEL to be used taking into account the quality of the studies, the number of animals, consistency of the observations, and the total context of the evidence. For the single dose studies, the time of dosing during pregnancy is a critical factor in view of the most sensitive window of exposure. Because most of the single dose studies were performed during the critical window of sensitivity or were designed to establish that window, we normally used the day of treatment that provides the highest incidence of adverse effects.

Throughout this report the NOAELs and LOAELs from the single dose studies are expressed as a percentage of the NOAELs and LOAELs from the repeated dose studies. So, percentage results represent NOAEL-NOAEL or LOAEL-LOAEL comparisons. However, this comparison alone is not sufficient. For example, $NOAEL_{single}$ and the $LOAEL_{single}$ can both be 300% of their corresponding repeated values but the $NOAEL_{single}$ may still be lower than the $LOAEL_{rep}$ depending on the choice of dosages in the studies. In that case, there may not be a true difference between single and repeated dosing. Therefore, we also calculated the ratio between the $NOAEL_{single}$ and the $LOAEL_{repeated}$ since a meaningful difference between single and repeated doses is only identified when no effects occur in a single dose study at levels that do induce effects in a repeated study. The $NOAEL_{single}$ / $LOAEL_{rep}$ ratio is abbreviated as NLR in this report.

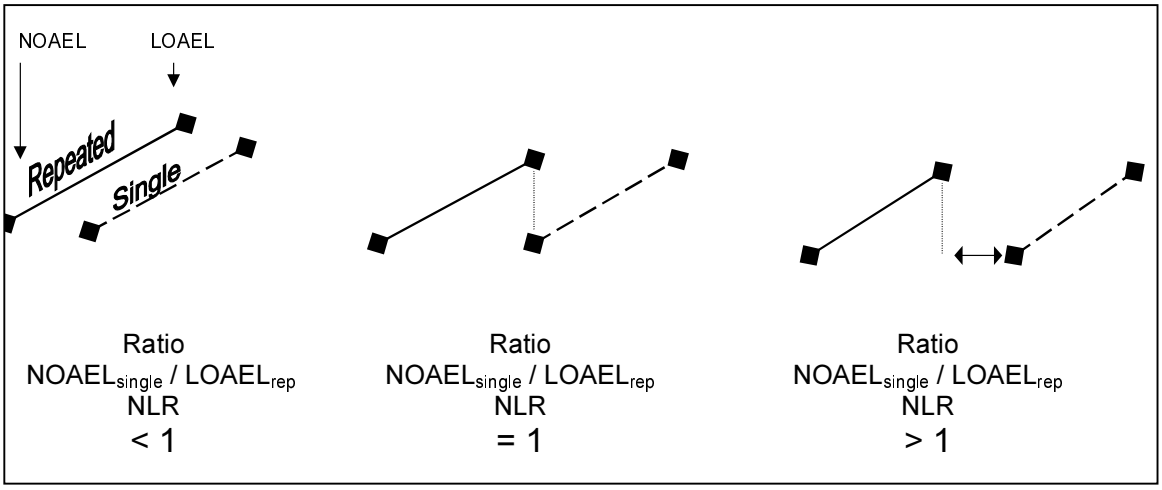

Figure 2. Schematic representation of repeated and single dose response curves, and the consequence for the $NOAEL_{single}$ / $LOAEL_{rep}$ ratio (=NLR). When NLR > 1, there is a true difference between the single and the repeated dose response curve.

So, the NRL provides two types of information. First, an NRL > 1 confirms that there is a true difference in the NOAELs of single and repeated dose studies. Second, the NRL provides and indications how large the difference is between single and repeated dosing.
The percentage and NLR results are present for each substance-species-route combination separately and in addition, mean values ± S.D. are given for the total set of data. No further statistical analyses were performed. Primary data for maternal toxicity, resorptions, fetal body weight, and number of fetuses with malformations/variants (percentage and NLR calculations are given in Appendix A.
Unfortunately, not all studies available studied or reported the total range of developmental toxicity parameters addressed in this investigation. Therefore, for some endpoints only very limited information is available. In this report we will focus on the following endpoints for which a substantial amount of information was available: maternal toxicity, embryonal or fetal resorptions, fetal body weight, the percentage of abnormal fetuses, and skeletal defects.

3. Results

Information for single or short term exposures were found for the substances listed in ***Table 1***. The species and route of exposure are included. All the individual data for each substance as well as references to the original publications used are provided in Appendix B.

Table 1. Overview of available data.

Substances	Species	Route	Repeated dose available	Single dose available
Arsenic trioxide	rat	oral-gavage	yes	Yes
Benomyl		oral-gavage	yes	Yes
Bropirimine		oral-gavage	yes	Yes
Butyl Benzyl Phthalate	rat	oral-gavage	yes	Yes
Cadmium	mice	Sc	Yes, oral gavage	Yes
Cadmium	mice	Ip	Yes, oral gavage	Yes
Carbendazim	rat	Oral-gavage	Yes	yes
Carbon tetrachloride		oral-gavage	Yes	Yes
Chlorpropham	mice		yes	Yes
Di-n-butylphthalate	rat	oral-gavage	Yes	Yes
ETU	rat	oral-gavage	Yes	Yes
ETU	hamster	oral-gavage	Yes	Yes
Methanol	rat	oral-gavage	yes	Yes
Methanol	rat	Inhalation	Yes	No
Methanol	mice	inhalation	Yes	Yes
2-Methoxyethanol	rat	oral-gavage	Yes	Yes
2-Methoxyethanol	mice	oral-gavage	Yes	Yes
2-methoxyethanol	rat	dermal	Yes	Yes
Nickel	rat	i.m.	Yes	Yes
Paclobutrazole		oral	Yes	Yes
Phenytoin	rat	oral-gavage	Yes	Yes
Retinyl palmitate	rat	oral-gavage	Yes	Yes
Thiabendazole	rat	oral	Yes	No
Thiabendazole	mice	oral-gavage	Yes	Yes
Tributyltin (Cl, O)	rat	oral-gavage	Yes	Yes
Trichloroethylene	rat	oral-gavage	Yes	Yes

Although single dose and repeated dose studies were available, not all studies included a report on the full range of developmental toxicity endpoints. Therefore, sometimes information on only one specific endpoint (e.g. full-litter resorptions) was available while for a range of other endpoints (e.g. fetal body weight and skeletal effects) information was lacking. Therefore the number of substances for which ratios are available are somewhat

variable depending on the endpoint. NOAEL and LOAEL result tables as well as references to the studies used can be found in Appendix A.
In the following sections, the results for a number of specific endpoints are discussed.

3.1 General

From the results obtained it can be observed that without any doubt, a single exposure to a chemical substance is able to induce a wide range of different adverse effects on the pregnant animal and the developing foots. This includes maternal toxicity (mostly measured as body weight gain reduction) and a range of developmental endpoints such as fetal body weight, resorptions, fetal death, retarded ossification, malformations, and variants. In the next, paragraphs these type of endpoints will be further described. In principle all developmental endpoints investigated should be considered relevant for acute limit setting. However, the probability that a specific effect occurs at the same dose on a single day compared to a 'normal' guideline-based repeated exposure study (basically an OECD 414 study) may differ for the various endpoints as well as chemical substances.

3.2 Maternal Toxicity

Maternal toxicity is often only investigated by gross parameters such as food consumption and body weight gain reduction. Sometimes, also organ weights or specific physiological disturbances have been measured, especially in mechanistic studies. In this inventory, maternal toxicity is based only gross parameters (body weight, food intake, organ weights); no differentiation has been made between the parameters measured. A NOAEL for maternal toxicity has been established at the highest dose at which the original publications reported no effects on the dams. However, in the majority of publications used, maternal toxicity was represented by body weight and/or food consumption effects.
The primary data for maternal toxicity are presented in Appendix A. In figure 3 the NOAELs and LOAELs from single dose experiments ($NOAEL_{single}$ and $LOAEL_{single}$ respectively) are expressed as percentages of the NOAEL and LOAELs from the repeated dose experiments ($NOAEL_{rep}$ and $LOAEL_{rep}$ respectively). Essentially, a value of 100% indicates that the NOAEL in a single dose study is equal to the NOAEL of a repeated dose study. Only comparisons for which both the NOAELs and LOAELs are available yield valid comparisons. This holds true for 11 substances (Arsenic trioxide, BBP, Cadmium, Carbendazim, Deltamethrin, Methanol (oral-rat, inhal-mouse), 2-ME (dermal-rat), Phenytoin, and Thiabendazole). On average the $NOAEL_{single}$ is 751 ± 900 % of the $NOAEL_{rep}$ while the $LOAEL_{single}$ is 557 ± 552 % of the $LOAEL_{rep}$ and for most substances the values are ≥ 200%. This indicates that for the database studied on average a 5-fold higher dose is needed on a single day to induce maternal toxicity compared to the dose used in the repeated experiment.

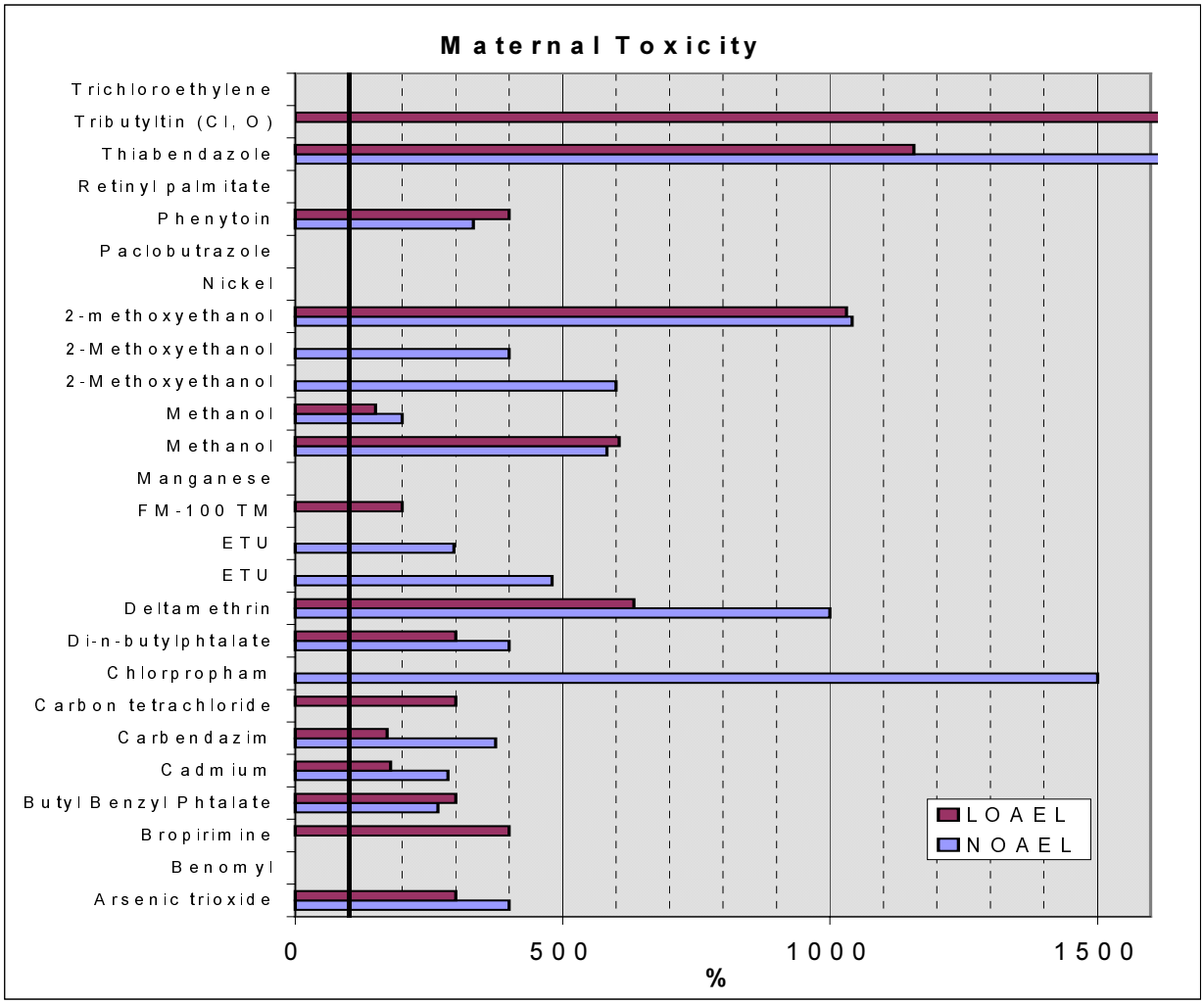

Figure 3. Single dose and repeated dose comparison for maternal toxicity. Results represent the $NOAEL_{single}$ and $LOAEL_{single}$ values expressed as percentage of the corresponding NOAEL and LOAEL values from repeated dose experiments. The solid line marks the 100% (i.e. no difference) line.

Table 2. NLR results for maternal toxicity

Substance	**NLR-value**
Carbendazim	NLR < 1
Cadmium, Methanol (inhal-mice), Phenytoin.	1 ≤ NLR < 2
Arsenic trioxide, BBP, DBP, Methanol (oral-rat), 2-ME (oral-mice),	2 ≤ NLR < 3
Chlorpropham, deltamethrin, ETU (oral-rat), 2-ME (oral-rat), 2-ME (derm-rat), Thiabendazole	NLR ≥ 3

The NLR is on average 3.01 ± 2.26, which confirms that there is a true difference between the NOAELs from single dose studies and the NOAELs from repeated dose studies (***Table 2***). It should be realized that this ratio is highly dependent on the dose levels chosen in the experiments. However, for those substances-species-route combinations for which this NLR could be calculated (n=14), the results are given in categories in ***Table 2***. The small ratio for carbendazim was based on an uncorrected decrease in maternal body weight gain in the single dose study (Minta et al., 1982) while post-implantation loss and the number of pups per litter decreased with dose. When maternal body weight gain would have been corrected for gravid uterus weight the $NOAEL_{single}$ would probably have been higher.
From these results it appears that the dose levels that induce maternal toxicity in a repeated developmental experiment (i.e. normal guideline-based study) are not representative for a single exposure. Maternal toxicity is apparently aggravated by repeated exposure to the test substance over a number of days. The use of a NOAEL for maternal toxicity from a repeated experiment is therefore highly conservative in view of acute toxicity limit setting.

3.3 Resorptions

The primary data of the analysis for resorptions is presented in Appendix A. In figure 4 the NOAELs and LOAELs from single dose experiments are expressed as percentage of the NOAEL and LOAELs from the repeated dose experiments. Essentially, a value of 100% indicates that the NOAEL in a single dose study is equal to the NOAEL of a repeated dose study. Only comparisons for which both the NOAELs and LOAELs are available yield valid comparisons. This holds for 14 substances (Arsenic trioxide, Bbenomyl, BBP, Carbendazim, ETU, MeOH, 2-ME, Nickel, Phenytoin, Thiabendazole, and Trichloroethylene). The figure shows that only for Benomyl and Phenytoin both the NOAEL and LOAEL comparison shows a value around 100% indicating no difference between the NOAELs of single and repeated dosing studies. However, for the other substances, the NOAELs and LOAELs of the single dose studies are about 200 – 400 % of the repeated dose values indicating 2-4 fold higher dosages are needed in a single day study to induce resorptions. On average the $NOAEL_{single}$ is 391 ± 440 % of the $NOAEL_{rep}$; the $LOAEL_{single}$ is 399 ± 486 % of the $LOAEL_{rep}$.

The mean NLR is 2.12 ± 2.23. The NLR results are presented as categories in ***Table 3***. These results confirm that for most substances, there is a true difference between the NOAELs in single dose and repeated dose studies.

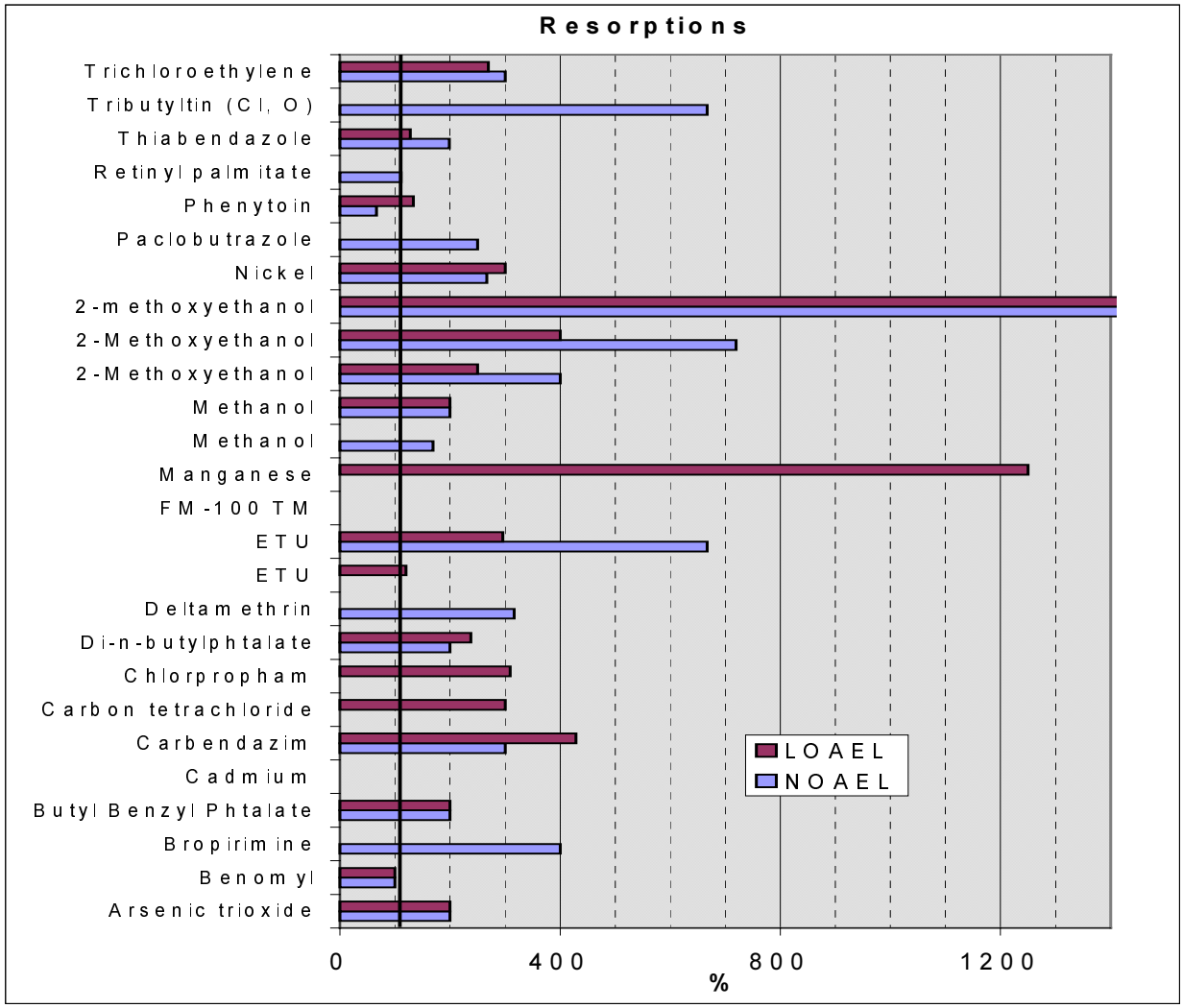

Figure 4. Single dose and repeated dose comparison for resorptions. Results represent the $NOAEL_{single}$ and $LOAEL_{single}$ values expressed as percentage of the corresponding NOAEL and LOAEL values from repeated dose experiments. The solid line marks the 100% (i.e. no difference) line.

Table 3. NLR results for resorptions

Substance	**NLR-value**
Benomyl, phenytoin	NLR < 1
Arsenic trioxide, BBP, Carbendazim, Chlorpropham, DBP, Methanol (oral-rat), Methanol (inhal-mice), Thiabendazole	1 ≤ NLR < 2
Bropirimine, ETU (oral-hamster), 2-ME (oral-rat), Nickel, Trichloroethylene	2 ≤ NLR < 3
2-ME (oral-mice), 2-ME (derm-rat),	NLR ≥ 3

With respect to resorptions, the evidence indicates that some substances induce resorptions after a single exposure at the same dose level as after repeated exposure although for most substances about higher doses are needed.

3.4 Fetal body weight

The primary data of the analysis for fetal body weight is presented in Appendix A. In figure 5 the NOAELs and LOAELs from single dose experiments are expressed as percentage of the NOAEL and LOAELs from the repeated dose experiments. Essentially, a value of 100% indicates that the NOAEL in a single dose study is equal to the NOAEL of a repeated dose study. Only comparisons for which both the NOAELs and LOAELs are available yield valid comparisons. This holds for 12 substances (Arsenic trioxide, Benomyl, BBP, Carbendazim,

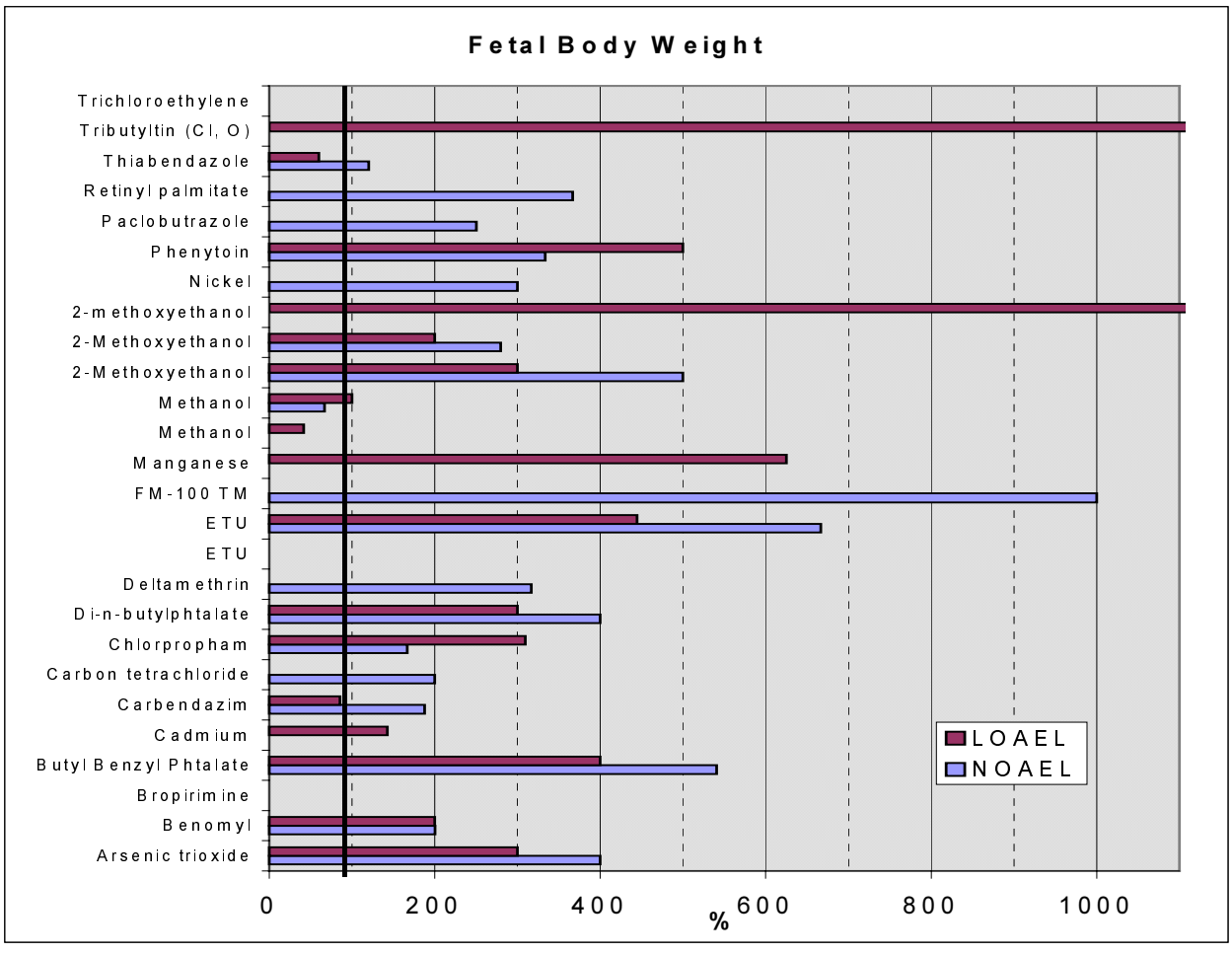

Figure 5. Single dose and repeated dose comparison for fetal body weight. Results represent the $NOAEL_{single}$ and $LOAEL_{single}$ values expressed as percentage of the corresponding NOAEL and LOAEL values from repeated dose experiments. The solid line marks the 100% (i.e. no difference) line.

Chlorpropham, DBP, ETU (oral hamster), MeOH (inhal-mouse), 2-ME (oral-mouse, oral-rat), and Thiabendazole). The figure shows that for Methanol and Thiabendazole, the NOAEL and LOAEL comparison shows a value around 100% indicating no difference in NOAELs between single and repeated dosing. However, the Methanol result is dominated by observations from a single exposure on day 5, which is not included in the normal repeated dose study which starts at day 6. In addition, the experiment yielding the low effect levels for the single dose experiments did not include a concurrent control group. Effects had to be compared to other control groups in the same publications which may have induced some bias. For Thiabendazole, the $NOAEL_{single}$ is determined from a single set of experiments showing fetal body weight effects at 60 mg/kg bw, while the same publication also contained a second set of single dose experiments which showed no effects at fetal body weight up to 129 mg/kg bw (same strain dosed on the same GD). So, the $NOAEL_{single}$ and $LOAEL_{single}$ for Thiabendazole could be about 2-fold higher depending on the interpretation of the primary data. When the higher NOAEL would be used, the $NOAEL_{single}$ and the $LOAEL_{single}$ would be 240 and 120% of the repeated NOAEL values respectively. For the other substances, the NOAELs and LOAELs of the single dose studies are about 200 – 400 % of the repeated dose values indicating 2-4 fold higher dosages are needed in a single day study to induce a decrease in fetal body weight. On average the $NOAEL_{single}$ is 350 ± 223 % of the $NOAEL_{rep}$; the $LOAEL_{single}$ is 476 ± 611 % of the $LOAEL_{rep}$.
The mean NLR is 2.30 ± 2.46. In ***Table 4*** the NLR results are presented in categories. For most substances the NLR is > 1 which confirms a true difference between NOAELs from single and repeated dose studies

Table 4. NLR results for fetal body weight.

Substance	**NLR-value**
Carbendazim, Methanol, Thiabendazole	NLR < 1
Benomyl, Cadmium, Chlorpropham, 2-ME (oral-mice).	1 ≤ NLR < 2
Arsenic trioxide, BBP, DBP, ETU (oral-hamster), 2-ME (oral-rat), Phenytoin,	2 ≤ NLR < 3
FM-100, 2-ME (derm-rat)	NLR ≥ 3

3.5 Percentage fetuses with Malformations or Variations

The primary data of the analysis for malformations and variants is presented in Appendix A. In figure 6 the NOAELs and LOAELs from single dose experiments are expressed as percentage of the NOAEL and LOAELs from the repeated dose experiments. Essentially, a

value of 100% indicates that the NOAEL in a single dose study is equal to the NOAEL of a repeated dose study.

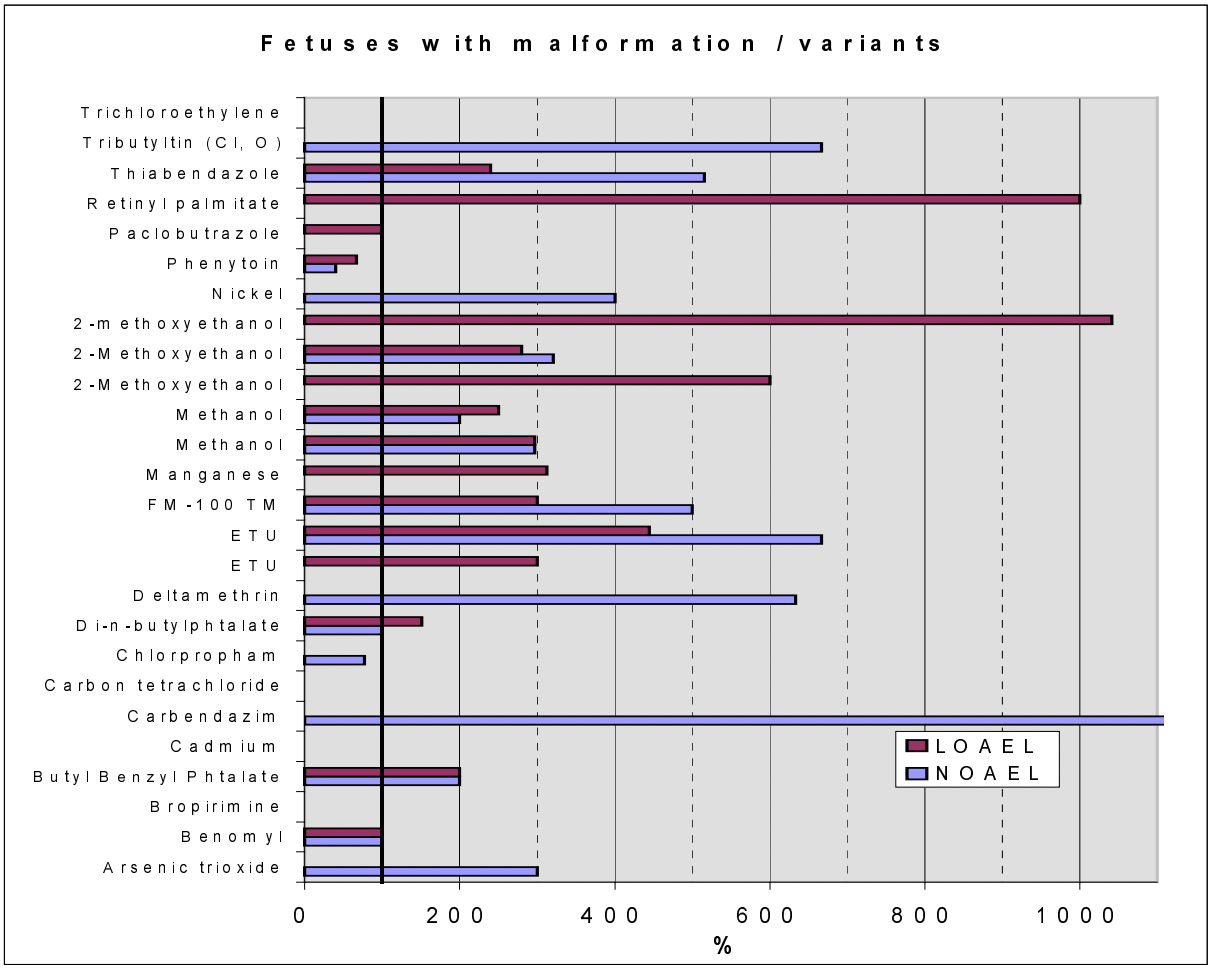

Figure 6.. Single dose and repeated dose comparison for fetal body weight. Results represent the $NOAEL_{single}$ and $LOAEL_{single}$ values expressed as percentage of the corresponding NOAEL and LOAEL values from repeated dose experiments. The solid line marks the 100% (i.e. no difference) line.

Only comparisons for which both the NOAELs and LOAELs are available yield valid comparisons. This holds for 11 substances (Benomyl, BBP, Carbendazim, DBP, ETU (oral hamster), FM-100, MeOH (inhal-mouse, oral-rat), 2-ME (oral-mouse), Phenytoin and Thiabendazole. The figure shows that for Benomyl, DBP, and Phenytoin, the NOAEL and LOAEL comparison shows a value around 100% indicating no difference in NOAELs between single and repeated dosing (***Table 5***). The fact that for these substances the NLR is lower than 1 confirms the absence of any difference between single and repeated dosing. However, for the other substances, the NOAELs and LOAELs of the single dose studies are

about 200 – 300 % (or more) of the repeated dose values indicating 2-3 fold higher dosages are needed in a single day study to induce an increase in the number of fetuses with malformations and/or variants. On average the $NOAEL_{single}$ is 431 ± 440 % of the $NOAEL_{rep}$; the $LOAEL_{single}$ is 355 ± 292 % of the $LOAEL_{rep}$.

Table 5. NLR results for the number of fetuses with malformations / variants.

Substance	NLR-value
Benomyl, DBP, Phenytoin,	NLR < 1
BBP, Methanol (oral-rat), Methanol (inhal-mice), 2-ME (oral-mice), Thiabendazole,	1 ≤ NLR < 2
ETU (oral-hamster), FM-100,	2 ≤ NLR < 3
Carbendazim, Deltamethrin, 2-ME (derm-rat), 2-ME (oral-rat), Retinyl palmitate,	NLR ≥ 3

The mean NLR is 2.37 ± 1.80. The NLR results are presented in categories in ***Table 5***. These results show that beside the 3 substances with a NLR < 1 (Benomyl, DBP, Phenytoin), there are 5 additional substance-species-route combinations with a limited difference (NLR< 2) Only 2 combinations have 2 ≤ NLR < 3. However, 5 combinations have a NLR ≥ 3 indicating a large difference in NOAELs and LOAELs between the single and repeated dose experiments. It appears that there might be two sets of substances. One group for which no difference between the NOAEL/LOAELs in single dose and repeated dose experiments is observed or the difference is at least < 2 ; and another group of substances for which the difference is large.

3.6 Skeletal effects

Skeletal effects do frequently occur in developmental toxicity studies. Partly this may be a specific teratogenic effect but partly these effects are the result of fetal growth retardation or general embryo/fetotoxicity (see above). In ***Table 6***, substance-species-route combinations are presented for which NOAEL and LOAEL comparisons were available between single and repeated dose experiments. The skeletal effects in this analysis only include fused arches, fused vertebrae, fused sternebrae, fused ribs, wavy ribs, digital effects, and shortened ribs. Other (specific) effects, including delayed ossification, are excluded. Although this pooling of different effects might obscure any specific difference between separate effects, further splitting of effects yields a too limited number of combinations. Nevertheless, the results in ***Table 6*** are dominated by fused sternebrae and fused arches/vertebrae.

Table 6. Skeletal effects: NOAEL and LOAEL comparisons between single and repeated dose experiments & NLR values.

Substance	Species	Route	$NOAEL_{single}$ (% of $NOAEL_{rep}$)	$LOAEL_{single}$ (% of $LOAEL_{rep}$)	Ratio $NOAEL_{single}$ / $LOAEL_{rep}$
BBP	rat	Oral-gavage	-	133	-
Thiabendazole	mouse	Oral-gavage	17	18	0.09
Phenytoin	rat	Oral-gavage	133	200	0.7
2-Methoxy-ethanol	mouse	Oral-gavage	160	200	0.8
Methanol	mouse	Inhalation	200	250	1.0
Paclobutrazole	rat	Oral-gavage	400	250	1.0
DBP	rat	Oral-gavage	180	238	1.6
FM-100	rat	Inhalation	500	300	2.0
ETU	hamster	Oral-gavage	667	444	2.2
Deltamethrin	rat	Oral-gavage	633	-	3.2
Carbendazim	rat	Oral-gavage	1875	-	4.2
2-Methoxy-ethanol	rat	dermal	-	1042	5.2
Methanol	rat	Oral-gavage	1223	-	6.1

From this analysis, a similar picture appears as was anticipated in the preceding paragraph on malformations/variants. For some substances, the single dose is at least equally effective compared to the repeated experiment (Methoxyethanol-mouse, Phenytoin, Thiabendazole). However, for other substances a very clear difference can be observed between the single dose and the repeated dose NOAELs and LOAELs (Carbendazim, Deltamethrin, Methanol (oral rat), 2-Methoxyethanol (dermal rat)). There appears to be no difference in the type of skeletal effects that are induced. In the both group with low NLR and the group with high NLR a differential scope of skeletal effects is present (primarily fused vertebrae, arches or ribs). Large differences between individual substance-species-route combinations are likely based on differences in maternal toxicity, mechanism of action or kinetic differences, or a combination of these. It is, however, beyond the scope of this study to evaluate specific kinetics/metabolism or mechanisms of action on a detailed substance specific level.

With respect to retarded ossification, only limited information was available for 5 substance-species-route combinations (***Table 7***). For 4 substances, a substantial difference was observed in NOAELs / LOAELs for retarded ossification between single and repeated exposure. Only for phenytoin, no difference could be established (NOAELs and LOAELs about the same and the ratio $NOAEL_{single}$ / $LOAEL_{rep}$ was 0.44. Unfortunately, the information is too limited to provide general conclusions based on these data.

Table 7. Retarded ossification: NOAEL and LOAEL comparisons between single and repeated dose studies & NLR results.

Substance	Species	Route	$NOAEL_{single}$ (% of $NOAEL_{rep}$)	$LOAEL_{single}$ (% of $LOAEL_{rep}$)	Ratio $NOAEL_{single}$ / $LOAEL_{rep}$
Phenytoin	rat	Oral	67	133	0.44
Methanol	rat	Oral	-	-	1.7
ETU	hamster	Oral	667	444	2.2
FM-100	rat	Inhalation	1000	-	4.0
2-Methoxy ethanol	rat	Dermal	-	1042	5.1

3.7 Specific Malformations

For some substance-species-route combinations data were available to compare the NOAELs and LOAELs from single dose studies with those of repeated studies. However, the number of combinations for which a complete NOAEL/LOAEL comparison was possible was rather limited (***Table 8***). Results have been obtained for cleft palate, exencephaly, and dilation of the renal pelvis (including hydronephrosis).

Table 8. Specific malformations: NOAEL and LOAEL comparisons between single and repeated dose studies & NLR results.

Substance	Species	Route	$NOAEL_{single}$ (% of $NOAEL_{rep}$)	$LOAEL_{single}$ (% of $LOAEL_{rep}$)	Ratio $NOAEL_{single}$ / $LOAEL_{rep}$
Cleft Palate					
Methanol	mouse	Inhalation	250	200	1.0
BBP	rat	Oral-gavage	200	200	1.3
DBP	rat	Oral-gavage	180	238	1.6
Retinyl palmitate	rat	Oral-gavage	550	333	1.8
ETU	hamster	Oral-gavage	667	444	2.2
Exencephaly					
Retinyl palmitate	rat	Oral-gavage	333	333	1.11
Dilation of renal pelvis (incl. Hydronephrosis)					
Phenytoin	rat	Oral-gavage	133	200	0.7
DBP	rat	Oral-gavage	-	300	2.0
Carbendazim	rat	Oral-gavage	1875	-	4.3
Neural tube defects					
Methanol	mouse	Inhalation	-	100	-

From this table, it appears that for most substance-species-route combinations a higher dose is needed in a single exposure to induce the specific malformation compared to a repeated

exposure experiment (NOAELs and LOAELs are all > 100%). Although the NLR is > 1 for most case (except for Phenytoin) indicating a difference between single and repeated dosing, the NLR is only about 1-2 (except for Carbendazim). The difference in dose response curves for specific malformations appears to show a limited difference between the dosages in single and repeated exposures. However, similar to the situation with retarded ossification, the available information is too limited to draw general conclusions.

3.8 Other indications for acute vs. repeated effects

Besides the comparison of NOAELs and LOAELs from repeated and single dose developmental studies, also other types of information can be found that gives insight in the importance of exposure duration for the induction of effects. For a number of substances, additional information was found and is presented in the next paragraphs.

FM-100 Flame retardant (inhalation studies in rats)

Results on the developmental toxicity of FM-100 were provided in two abstract publications by Nemec and collegues (1992a, b). The results are summarized in Appendix B where a 6h exposure on GD 9 was compared to daily 6h exposures during GD 6-15. In addition, also single 2h exposures were investigated. The results for this substance with respect to fetal body weight and skeletal variations is given in ***Table 9***.

For maternal toxicity, fetal body weight, and the induction of skeletal variations, a clear dose-time-response relationship appears. The NOAEL levels of the shorter durations (2h single vs. 6h single, and 6h single vs. 6h repeated) appear to be consistently higher than the effect levels in the longer durations. For example: skeletal effects are induced at 10,000 ppm in the repeated experiment but not at 20,000 ppm in the single 6h experiment. Similarly, skeletal effects were noted at 30,000 ppm for 6h but no effects are observed up to 60,000 ppm for 2h.

Table 9. Developmental toxicity of FM-100 after inhalatory exposure (Nemec et al., 1992a,b).

	Repeated study 1000, 4000, 10000 ppm (6h/day) GD 6-15	Single dose study 20000, 30000, 40000 ppm, 6-hours on GD 9	Single dose study 20000, 40000, 60000 ppm, 2-hours on GD 9
Maternal toxicity	BW and Food intake at ≥ 10,000 ppm	Reduced BW gain, CNS effects at ≥ 20,000 ppm.	CNS effects at ≥ 20000 ppm. Reduced BW gain at ≥ 40,000 ppm
Fetal body weight	Reduced at 10,000 ppm. No effect at 4000 ppm	No effect on fetal BW	No effect on fetal BW
Skeletal variations #	Present at ≥ 10,000 ppm. No effect at 4000 ppm	Present at ≥ 30,000 ppm. No effect on 20,000 ppm	No effect on skeletal parameters (≤ 60,000 ppm)

7^{th} cervical rib (in repeated dose and single 6h study), retarded ossification

2-Methoxyethanol (Studies in mice)

In a paper by Horton et al. (1985) oral exposures to 2-ME were performed in 3 days, 2 days, and single day exposures. In figure, the most important results are presented.

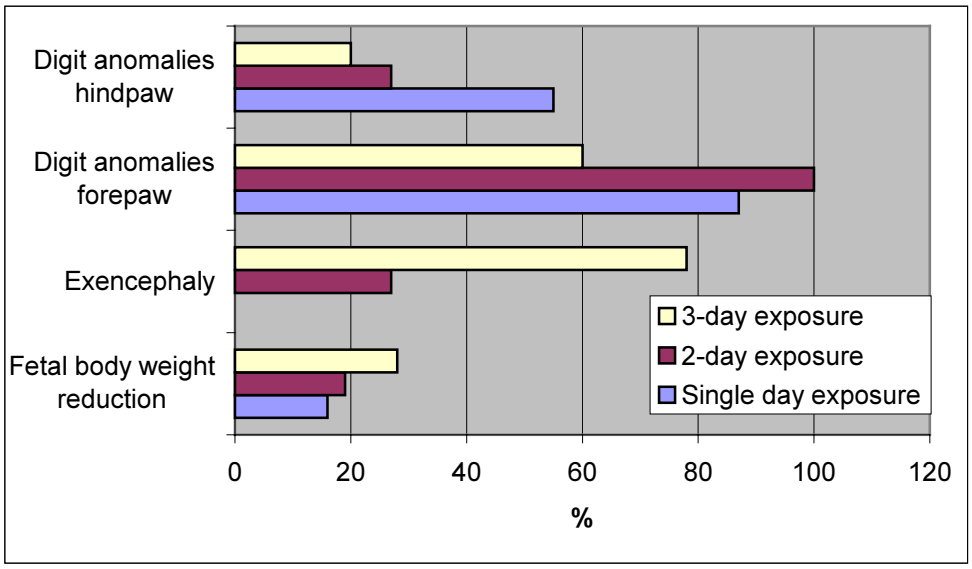

Figure 7. Comparison of single -, 2-day, and 3-day exposures to 2-ME at a dose of 250 mg/kg bw/day (oral gavage in mice). Effects are expressed as percentage reduction compared to controls (fetal BW) or as percentage litter incidence (taken from Horton et al., 1985).

For fetal body weight and exencephaly there is a correlation between the extent of the effect and the number of days of exposure, indicating that repeated exposure within the same period aggravates the induced effect (figure 7). For digit anomalies, such a correlation apparently does not occur. For this type of developmental toxicity, a single dose might be equally or more effective compared to 2- or 3-day exposures. However, in this experiment the digit anomalies were determined in a 2-day experiment at GD 10-11 or 9-10, and in a 3-day experiment at GD 9-11. The single day experiment with 250 mg/kg bw, however, was performed only on GD 11. In this case, the repeated experiments were started on an earlier gestation day compared to the single dose study. 2-ME is being metabolized by alcohol dehydrogenase (to form the teratogenic metabolite 2-methoxyacetaldhyde) and aldehyde dehydrogenase (to remove the metabolite) which are both easily inducible enzymes. Therefore, it is possible that in the 2- and 3-day experiments, metabolism has been induced resulting in a lower internal exposure level at the critical days for digit anomalies (day 11) compared to a single dose at day 11 only.

In another study by Terry et al. (1994), single day and 2 day exposure were used by i.v. injection using the same dose as Horton et al.. In figure 8, the main results are shown. As can

be seen in figure 8, repeated exposure to the same dose (250 mg/kg bw/day) induces a substantially higher effect on fetal body weight and on the incidence of exencephaly than a single dose. In this respect, it should be noted also that a singles dose up to 450 mg/kg bw did not yield a reduction in fetal body weight as large as a 3 day exposure at 250 mg/kg bw/day (Terry et al., 1994). The maximal reduction in fetal body weight with a single dose up to 450 mg/kg bw is 19% while the reduction is 28% after 3 days at 250 mg/kg bw. Unfortunately, no data were provided for the incidence of exencephaly at a single dose of 250 mg/kg bw. In this study, a similar profile is found as found by Horton et al. (1985). Increased incidences are observed for reduction in fetal body weight, exencephaly and resorptions in the 2-day exposures compared to the single day exposures. However, at the dose used (250 mg/kg bw i.v. injection) all litters had fetuses with skeletal malformations (rib and vertebral malformations) either in single day or 2-day exposures.

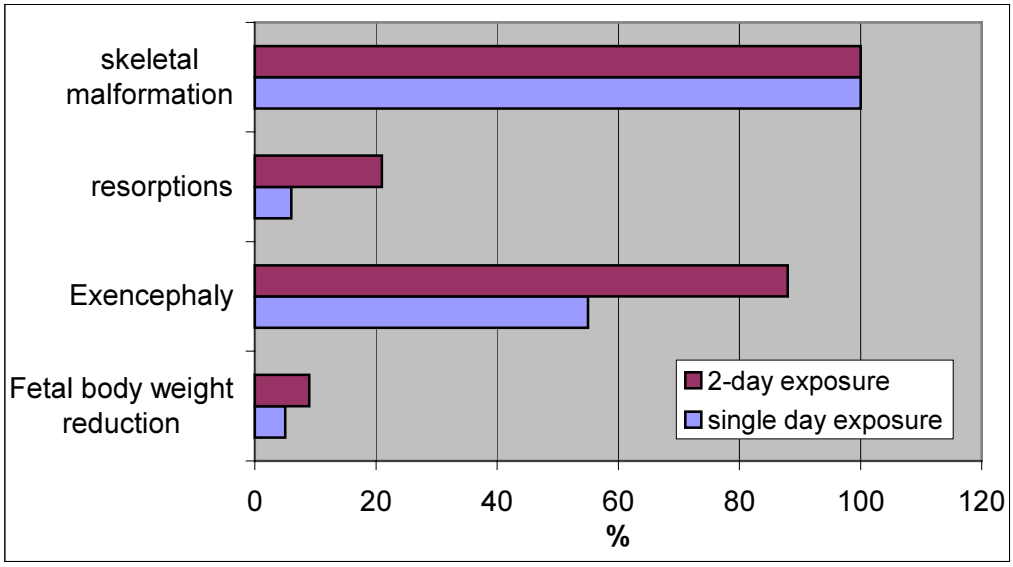

Figure 8. Comparison of single - and 2-day exposures to 2-ME at a dose of 250 mg/kg bw/day (iv injection in mice). Effects are expressed as percentage reduction compared to controls (fetal BW) or as percentage litter incidence (taken from Terry et al., 1994).

Bromodichloromethane (oral-gavage F-344 rats)

In an abstract publication by Narotsky et al. (1993) they reported the incidence of full-litter resorption after 2, 4, 7, and 10 days exposure to 75 mg/kg bw/day (GD 6-7, 6-9, 6-12, 6-15). Full-litter resorption incidence was 12%, 48%, 50%, and 65% for these four exposure periods respectively, compared to 0% for controls. Although the 2-day exposure might lack the inclusion of the most sensitive day for full-litter resorption (possibly around GD 9 for this substance), the window of sensitivity is most likely larger than one day. Nevertheless, the

results indicate that there is a continuum of risk associated with exposure duration, rather than a discrete critical period for this effect.

Methanol (Inhalation studies in mice)
In two sets of experiments (Bolon et al., 1993 and Rogers and Mole, 1997) single day and 2-day inhalation exposures were performed at concentrations of 10000 and 15000 ppm. However, in the experiments by Rogers and Mole, the single day experiments had no concurrent control groups which hampers a valid single-two-day comparison. So, in figure 9 only the main findings from Bolon et al. (1993) are shown.

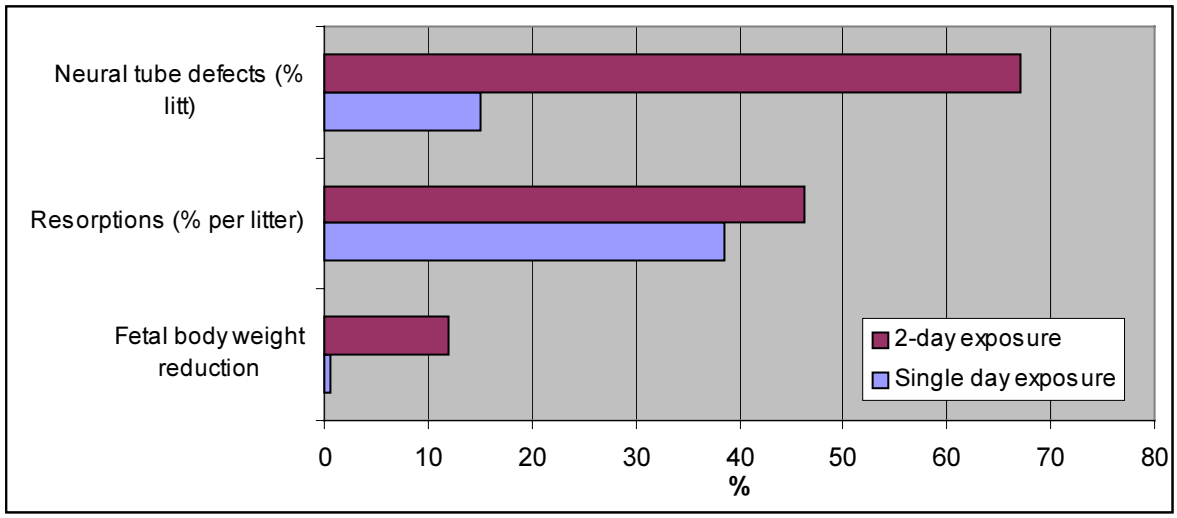

Figure 9. Comparison of single - and 2-day inhalatory exposures to Methanol at a concentration of 15000 ppm (7h)of mice. Effects are expressed as percentage reduction compared to controls (fetal BW) or as percentage litter incidence (Taken from Bolon et al., 1993).

These results show that fetal body weight is not affected by a single day exposure but is suppressed by 12% in a two day exposure. The incidence of neural tube defects increases substantially in the 2-day exposures. In contrast, little difference between single and 2-day exposures is observed for the occurrence of fetal resorptions.

4. Discussion

The effects of acute exposure to chemicals has become a more important activity in the risk assessment activities of RIVM / SIR. For some time, SIR has been involved in the risk assessment in cases of exceeding of health-based limit values, mostly limits for chronic exposure. In addition, in the last decade some activities have been introduced which primarily focus on the public health risk of acute exposure to substances. This includes risk assessment of consumer products and biocides with low frequency use, the setting of ARfD's for pesticides, and the setting of Emergency Response guideline values for hazardous substances. With respect to the latter activity, SIR participates in the setting of Dutch Intervention Values for Hazardous Substances and the Acute Exposure Guideline Levels (AEGL)-program of the US-EPA.

Although health-based limit values have been set as preventive limit values, acute limits appear to have or develop less preventive characteristics. To illustrate, the ARfD has been introduced as a tool for the evaluation of acute toxic effects of pesticides in the regulatory framework. However, the ARfD is more and more becoming a tool for actual risk assessment when pesticide residue limits have been exceeded. In addition, AEGLs are defined as threshold values above which a more serious type of effects will occur within the population. As such AEGLs are not purely preventive values but are guidelines to predict at what level a certain type of effect will occur. This tendency requires limit values for acute exposure that are based on appropriate and relevant endpoints and associated NOAELs without an overload of conservatism.

Developmental toxicity has been regarded to be highly relevant for acute limit setting because of the limited dosing period used in these studies (Dewhurst, 2000, Billington and Carmichael, 2000). As a default assumption, any effect on the foots has been considered relevant for acute limit setting. To illustrate this importance, about 15-30% of the ARfD's now available have been set on the basis of developmental toxicity (depending on the organization or country involved). In addition, for about 10% of the substances with Dutch Intervention Values for hazardous substances (1 hour emergency response values) the AGW-values[†] have been set on the basis of developmental or reproduction toxicity endpoints. As an extra aspect, some organizations consider developmental effects to be very severe such that extra modifying safety factors are applied.

However, one should consider that a normal 'guideline-based' developmental toxicity study in rats or rabbits involves a 10-14 day exposure on subsequent days. Although in comparison to most other studies in a 'standard' database this exposure period is relatively acute, this experimental treatment should be considered relatively 'repeated' in view of the limits that will be set on the basis of these effects. For example, a 10 day exposure period in rats covers about 40-50% of the total gestation period. In contrast, the ARfD and AEGLs are limits for a single exposure lasting < 24h (ARfD) or ≤ 8h (AEGLs). A single day exposure of a pregnant woman covers less than 1% of the total human gestation period.

The ‘critical window’ concept in developmental toxicology considers that critical limited periods of time in gestation exist during which the conceptus is relatively sensitive for the induction for specific malformations (see figure 1). Exposure outside the window of sensitivity does not necessarily correlate to a zero risk. These windows are different for different malformations, both in timing and duration. In addition, repeated exposure outside the critical window may aggravate adverse effects and reduce the possibility of (partial) recovery.
For some effects, the NOAEL observed in a repeated dose study may actually be determined by effects that are correlated to a critical window and hence the NOAEL is predictive for a single day exposure. However, this concept is clearly not valid for all types of endpoints determined in developmental toxicity studies. Billington and Carmichael (2000), have suggested that embryo-fetal survival and malformations are effects which can be induced by a single dose given during the critical period of development, but this would not be the case for general parameters such as fetal- and maternal body weight.

In the present study, the potential difference between NOAELs and LOAELs in single dose studies and normal ‘guideline-based’ repeated dose studies has been investigated for several effects determined in developmental toxicity studies. Our hypothesis was that for some developmental effects the NOAELs and LOAELs in single dose experiments would be equal to the NOAELs and LOAELs in repeated dose studies, and therefore these NOAELs are relevant for setting acute limit values. When a specific effect rather requires – or is aggravated by – some form of repeated dosing, the NOAELs and LOAELs in such a study will be less relevant for setting acute limit values because in a single dose experiment the NOAEL would be actually higher. A true difference would be obtained if the $NOAEL_{single}$ would be clearly higher than the $LOAEL_{rep}$, i.e. the ratio of $[NOAEL_{single} / LOAEL_{rep}] \geq 1$ (NLR).
This analysis has been performed for 26 substance-species-route combinations. However, for most endpoints only data for about 15 combinations were actually available. In the section below, a list of limitations and questions are discussed to place this investigation in perspective.

4.1 Limitations of the analyses

Limited number of observations
The analysis is, for most endpoints, based on a very limited number of substances. In addition, some substances occur more than once in the analysis. ETU has been studied in rats and hamsters (both oral), methanol has been studied in rats (oral) and mice (inhalation), and 2-Methoxyethanol has been studied by the oral route in rats and mice and by the dermal route in rats. It is not clear at present how representative this analysis is for the whole range of chemical substances (see also below).

† AGW = Alarmeringsgrenswaarde (similar to AEGL-2)

Actual developmental toxicants
This investigation is dominated by established developmental toxicants and teratogens. This is because specialized single dose studies are mostly performed to define the critical window of sensitivity for a specific effect or to study the mechanistic actions of a substance. Implicitly this always involves substances that are acknowledged developmental toxicants and or teratogens. With substances that do not induce specific developmental such single dose studies will normally not be performed. This might be regarded as an actual worst case estimation for the whole range of chemical substances, since with other substances more differences might be expected between single and repeated dosing. However, it is unknown whether the picture that emerges from our investigation is representative for the whole range of chemical substances.

Dose regimens and effects
Because many of the single dose studies are focused on the critical window and / or the mechanism of action, most of these studies use a (pre-defined) dose that actually causes the effects. These studies have no objective of determining a dose-response relationship or a NOAEL. Furthermore, such studies are often focused on a single type of effect (e.g. full litter resorptions) instead of investigation of a whole package of endpoints. Because of these aspects, more than one single dose study is often needed with different dosages to obtain a valid picture of the dose response relationship for a single day exposure. However, for a number of combinations only one effect level was obtained (i.e. only a LOAEL) which limits this investigation.

Influence of Maternal Toxicity
Maternal toxicity is an important element in developmental toxicity, being the primary cause of a range of developmental effects. A direct relation between maternal toxicity and developmental effects is, however, difficult to establish. For a number of developmental effects a relation with maternal toxicity has been indicated (Khera 1984; Khera 1985; Kavlock et al., 1985) among which are some skeletal variants. Kavlock et al. (1985) studied the relation between acute maternal toxicity and developmental effects and showed a correlation between the occurrence of supernumerary ribs and maternal toxicity. For other parameters such a correlation was less evident or absent.
Khera (1984) showed within an analysis of mouse developmental studies that maternal toxicity was always found accompanied by resorptions and fetal body weight reductions. However, these effects were also observed without maternal toxicity. In a number of studies with maternal toxic dosages, a pattern of defects was observed which included exencephaly, open eyes, hemivertebrae, fused arches or vertebrae, fused sternebrae, and fused, missing, or supernumerary ribs. However, such effects may be observed also (with other substances) at maternally non-toxic dosages. So, it is not possible to identify specific effects that are

exclusively induced by maternal toxicity although maternal toxicity has probably an etiologic role in a number of the defects listed above.

Critical Window
An analysis as presented in this report is only valid as long as the single doses have been given within the critical window of sensitivity during organogenesis. This critical window may differ depending on the effect (see also above and figure 1). When single dosages have not been given at the most appropriate time, the effect of a single dose study will be underestimated. Most single dose studies were actually designed to establish the specific critical windows for specific effects. Moreover, another part of the single dose studies involved dosing at or within the critical window as already establish for a specific substance. For each effect separately, the data of the most sensitive day were used to provide the lowest NOAEL / LOAEL. Therefore, this analysis is not likely to be hampered by any substantial extent by underestimation of the effects of single dose studies. Furthermore, the critical window of sensitivity is probably not confined to one single day (see also figure 1). This also indicates that some repeated dosing within the window of sensitivity may result in a higher incidence of effects compared to a single dose within that window.

Gavage vs. diet and route specificity
This analysis is dominated by oral-gavage studies (18 of the 26 available combinations). Gavage dosing could be characterized as daily bolus dosing which provides another kinetic pattern within the body compared to dietary exposure. NOAEL and LOAEL values in dietary studies are in a number of cases higher than NOAEL and LOAEL values in corresponding gavage studies. With dietary exposure the substance is taken up more gradually often resulting in substantially lower maximal blood and tissue concentrations of the substance compared to gavage dosing. Therefore, one could question whether or not in this analysis, substance-species-route combinations for which no difference was observed between single and repeated dosing in fact involve C_{max} – related effects while for combinations with substantial differences AUC-related effects are involved.
A similar argumentation can be used for route specificity. Can the results obtained primarily by oral dosing be declared relevant for inhalation exposure and hence for acute intervention values such as AEGLs? Although the gross picture of relevance of effects is, in our opinion, relevant also for inhalatory exposure, there will always be substances that show e.g. route specific metabolism. However in terms of kinetics, inhalation exposure (often 4-8h per day) could be considered as an intermediate between gavage-dosing (bolus) and dietary exposure because inhalation exposure only accounts for a limited period during the day but compared to gavage-dosing the substance is taken up more gradually during inhalation.
Therefore, at present the results are considered relevant also for inhalatory exposure.

4.2 Indications and Conclusions

4.2.1 Maternal toxicity

The results of our analysis show that gross maternal toxicity – captured only by body weight changes, food intake, clinical signs and organ weights – in single day exposure is only induced at higher dosages compared to the standard repeated studies. On average the NOAELs and LOAELs are about 5-7-fold higher in single day exposures. Except for carbendazim, the NLR was always higher than the observed effect level in the repeated studies which confirms that there is a true difference in the NOAELs between single and repeated dose studies. In the case of carbendazim, maternal body weight in a single dose study was based on an uncorrected decrease in maternal body weight gain in the single dose study (Minta et al., 1982) while post-implantation loss and the number of pups per litter decreased with dose. When maternal body weight gain would have been corrected for gravid uterus weight the $NOAEL_{single}$ would probably have been higher. Methanol inhalation in mice showed the smallest difference in maternal toxicity NOAELs and LOAELs between single and repeated exposure, the single values being only 2-fold higher.
Based on these observations it is concluded that the NOAEL for gross maternal toxicity (body weight changes, food intake, clinical signs, organ weights) observed in a standard repeated dose developmental toxicity study is not an appropriate starting point for acute limit setting.
It should be emphasized that this is only applicable for gross toxicity endpoints as described and does not automatically apply to other specific effects measured in pregnant females. For example, cholinesterase inhibition by carbamates determined in the dams is a highly relevant endpoint for acute limit setting.

4.2.2 Resorptions

Resorptions are the consequence of mortality of the implanted embryo or fetus and are normally categorized in early and late resorptions. Because in normal guideline based studies dosing of the chemical starts shortly after implantation, especially early resorptions reflect the effect of a rather short dosing period (Van Raaij, 2001). Late resorptions per se occur only after a number of days exposure in regular studies. Thus, ideally a separate analysis should be made for early and late resorptions although the possibility that a single exposure later during organogenesis induces late resorptions cannot be excluded. In addition, most studies available for this investigation did not discriminate between early and late resorptions. Because of these reasons, resorptions are treated as one endpoint without differentiation in timing when they occur.
The results showed that on average the NOAELs and LOAELs of single day treatments were about 2-4 fold higher compared to the repeated values. For some substances however the NLR was < 1 indicating essentially no difference between single and repeated exposure (Benomyl and Phenytoin). For a substantial amount of substances the difference between single and repeated exposure was evident but limited: the NLR being < 2. Inhalatory

exposure to methanol in mice showed that at a similar dose, the extent of resorptions was somewhat higher in a two-day exposure compared to a single day exposure but the difference was limited (section 3.8 above). Although also larger differences were observed between single and repeated exposure (e.g. for 2-methoxyethanol (see ***Table 3*** and section 3.8 above)), this analysis indicates that resorptions can occur as a consequence of single exposures at about the same dose levels as those used in a standard type developmental study. The NOAEL for resorptions observed in repeated dose studies is thus considered to be a relevant starting point for acute limit setting.

4.2.3 Fetal Body weight

Fetal body weight (gain) reduction is often taken as a marker for fetal growth retardation. It has been postulated that fetal body weight reduction is a non-specific type of endpoint that would be more representative of repeated than of acute exposure (Billington and Carmichael, 2002). In addition, fetal body weight reduction is often related to the occurrence of maternal toxicity and as such considered as a less relevant effect for direct exposure of the fetus. In addition, temporary growth retardation induced on a specific day during gestation does not necessarily result in a decreased body weight at birth because slight growth retardation can be compensated during the remainder of the gestation period.
In the present analysis it is shown that for 2 substances, the NOAELs and LOAELs of single and repeated exposure were similar (Methanol and Thiabendazole). However, the MeOH result is dominated by observations from a single exposure on day 5, which is not included in the normal repeated dose study which starts at day 6. In addition, the experiment yielding the low effect levels for the single dose experiments did not include a concurrent control group. Effects had to be compared to other control groups in the same publications which may have induced some bias. In addition, in section 3.8 the 1-day and 2-day experiments with methanol inhalation in mice show that a 2-day exposure at 15000 ppm decreased fetal body weight but a single exposure at this dose did not affect fetal body weight. For Thiabendazole, the results are dominated by a single set of experiments that shows effects on fetal body weight at 60 mg/kg bw, while the same publication also contained an set of experiments showing no effects at fetal body weight up to 129 mg/kg bw (same strain dosed on the same GD). So, the $NOAEL_{single}$ and $LOAEL_{single}$ for thiabendazole could be about 2-fold higher depending on the interpretation of the primary data. Nevertheless, besides Methanol and Thiabendazole the NLR was also limited for Carbendazim (0.43) and Benomyl and Cadmium (about 1). For most other substances the NOAELs and LOAELs in single day experiments are about 2-4 fold higher compared to the values from repeated dose experiments. This indicates that the difference in NOAELs / LOAELs between single and repeated exposure is more variable for fetal body weight than for e.g. resorptions. For some substance-species-route combinations the NOAEL for fetal body weight reduction observed in a repeated dose study seems a relevant starting point while for other combinations such a NOAEL is not representative for a single exposure. The relevance of fetal body weight as endpoint for acute limit setting should therefore be evaluated within the total context of effects including maternal toxicity and other

fetal effects. For example, when fetal body weight reduction occurs only at doses that are maternally toxic and other effects on the fetus are limited to retarded skeletal ossification, the effects are probably caused by repeated exposure and are unlikely to occur from a single exposure at the same dose.

4.2.4 Number of fetuses with malformations / variants

The induction of irreversible structural effects in fetuses (malformations) occurs in most cases within a specific period of time during organogenesis. Each structure formation is initiated in a specific time window and its development occurs mostly over a limited period during gestation. For this type of effects often a critical window of sensitivity exists in which the fetus is very sensitive to exposure to a developmental toxicant. Therefore, it is anticipated that malformations can be induced by about the same dose level in repeated and single day experiments as long as the single dose is given within the critical window.
Based on the critical window concept the substance would only have to be present during a limited period of time to induce the effect. According to our hypothesis, no difference should therefore be observed between the NOAELs and LOAELs in single and repeated exposures. Although ideally one should analyze this hypothesis for each malformation separately, the available information is too limited to provide a valid analysis. Therefore, the number of fetuses with malformation or structural variants was taken as a gross parameter for this analysis because for this endpoint a NOAEL / LOAEL could be established for most endpoints.
For three substances (Benomyl, DBP, Phenytoin) no difference between single day and repeated exposure was evident ($NOAEL_{single}$ and $LOAEL_{single}$ were about 100% of the corresponding repeated values and the NLR was less then 1). Also for substances like Chlorpropham and Paclobutrazole possibly no difference occurs between single and repeated exposure (resp. NOAEL and LOAEL are similar). On the other hand, clear differences appear to occur for other substances (e.g. Carbendazim, Deltamethrin, 2-Methoxyethanol, Retinyl palmitate). When NLR results are considered there may appear two groups of substances: one group for which no major difference is evident (ratio < 2) and hence follows the critical window concept, and one group with clear differences (ratio > 3). This cannot be ascribed to malformations on the one hand and variants on the other because also for substances that induce clear malformations (e.g. Retinyl palmitate) a large difference can be observed between single and repeated exposure.
The differential picture that emerges for malformations and variants is likely based to other factors such as the kinetics of the substances (half-life, excretion, metabolism, build-up in tissues) and the mechanism of action (Cmax or AUC dependent; presence of substance necessary during an entire period to finally induce the effect; possibility for recovery). For example, retinoid teratogenesis has been shown to be related to the total AUC. In this case, it can be explained why a single dose has less effect than repeated dosing (Nau, 1990).

With respect to limit setting for acute exposures, normally such information is not available. Therefore it is concluded that malformations and major variants are – by default – relevant endpoints for acute limit setting unless specific information is available to indicate otherwise. In addition it should be emphasized that variants can occur in a wide variety of structures and severity while some variants (e.g. skeletal variants) occur also spontaneously and are often considered to be related to maternal toxicity. So, for variants the biological relevance of the effects in question always has to be taken into consideration when selecting endpoints for limit setting for acute exposures.

4.2.5 Skeletal effects

As indicated above, skeletal effects do frequently occur in low frequency (spontaneously or in relation to maternal toxicity) in developmental toxicity studies. The skeletal effects in the present analysis include fused arches, fused vertebrae, fused sternebrae, fused ribs, wavy ribs, digital effects, and shortened ribs. Although this pooling of different effects might obscure any specific difference between separate effects, further splitting of effects yields a too limited number of combinations. Nevertheless, the analysis is dominated by fused sternebrae and fused arches/vertebrae. Delayed ossification is excluded from this analysis because this effect is probably caused by other mechanisms (see below).
For skeletal effects, a similar type of picture emerges as was established for ‘number of fetuses with malformations and variants’. For some substances, no substantial difference could be established between single and repeated exposure while for others a large difference was observed. Most likely, similar factors contribute to this result as was discussed for malformation and variants in general (see above).
From this result, it is concluded that – by default – skeletal effects should be considered relevant endpoints for limit setting for acute exposures unless information is available to conclude otherwise. However, because skeletal variants such as fused ribs etc. occur also spontaneously and have been related frequently to maternal toxicity (Khera, 1984; Kavlock et al., 1982) the relevance of these type of endpoints should be carefully evaluated.

Unfortunately, little information on delayed or retarded ossification was available to draw any conclusions. Retarded ossification is sometimes observed at clear fetotoxic dosages but in some cases also at the lowest dosages, becoming the most sensitive effect in the study. In standard guideline-based studies with substances that are not specifically teratogenic or fetotoxic, retarded ossification is a very frequent occurring effect. The effect is often considered to be a parameter for general fetal growth retardation (Aliverti et al., 1979) and as such the parameter could be considered in a similar way like fetal body weight reduction. For fetal body weight reduction most NOAELs and LOAELs of single dose exposures are about 2-4 fold higher than the corresponding repeated values although also substance-species-route combinations were found where little difference was observed. Only for 5 substance-species-route combinations, a comparison was possible for retarded ossification. For 4 combinations, a substantial difference was observed between the NOAELs / LOAELs from a single

exposure and repeated exposures. Only for phenytoin, no difference was established (NOAELs / LOAELs are about the same; NLR was 0.44). At this moment, too little data are available to draw conclusions with respect to retarded ossification. As a first approach, one could propose to consider retarded ossification in a similar way as fetal body weight (see section 4.2.3). It should be noted that mild retarded ossification represents only some delay in normal development. So, the relevance of retarded ossification for acute limit setting should be considered within the total context of effects taking into account maternal toxicity and other effects on the fetus.

4.2.6 Specific malformations

Very little information was available in the present database to study the differences between single and repeated exposures for specific malformations separately. Only for cleft palate and dilation of the renal pelvis, single-repeated comparisons were possible for more than one substance. With respect to cleft palate, in general some difference in NOAEL and LOAEL values exists between single and repeated exposures. Of the 5 available substance-species-route combinations, 4 combinations had a NLR between 1 and 2. Although this indicates that repeated exposure probably has a more substantial influence on the induction of cleft palate than exposure on a single day, the differences are limited. Hence, cleft palate is considered a relevant effect for setting limits for acute exposures.
With respect to dilation of the renal pelvis (incl. hydronephrosis) a differential picture emerges: no difference for Phenytoin and a clear difference for Carbendazim with respect to single and repeated exposure. This difference is intermediate for DBP. Based on the observation with Phenytoin, dilation of the renal pelvis is a relevant endpoint for setting limits for acute exposures.
For neural tube defects, only information was available for Methanol (inhalation – mice). Although the $LOAEL_{single}$ was 100% of the $LOAEL_{rep}$, additional information shows a substantial difference in response at 15000 ppm between a single day and a two day exposure (see section 3.8 above). Nevertheless, this only indicates that repeated exposure results in a higher severity but the same dose actually still induces the effect.

4.3 General conclusions

In general the following conclusions can be drawn from this analysis:

- This study provides a limited set of data because for most endpoints studied only 10-15 substance-species-route combinations were available. Nevertheless, the present analysis is considered as a valuable starting point for evaluating the relevance of endpoints for limit setting for acute exposures.
- Because the analysis is based primarily on established teratogens and developmental toxicants, the results can be considered to represent a worst-case situation where the difference between single and repeated exposure are small. Substances with non-specific

effects on the developing fetus will probably show larger differences between single and repeated exposure.

- Gross maternal toxicity (body weight gain, food intake, clinical signs, organ weights) observed in standard guideline-based developmental toxicity studies is not relevant for setting limits for acute exposure because a clear difference in NOAELs / LOAELs is observed between single and repeated exposure. Specific acute maternal toxicity effects which are independent of the number of exposures (e.g. cholinesterase inhibition) are still relevant and should not be incorporated in this conclusion.
- The previous conclusions may have consequences for the evaluation of other effects also, in case they are considered to be the result of maternal toxicity. At this moment – and based on the present analysis – no clear guidance can be given how to discriminate between effects that may or may not be the consequence of maternal toxicity with respect to single exposures.
- Resorptions observed in standard guideline based developmental toxicity studies are considered to be relevant endpoints for setting limits for acute exposure because for most substances a limited difference is observed between single and repeated exposure.
- The relevance of fetal body weight reductions (and retarded ossification) for acute limit setting should be evaluated within the total context of effects taking into account maternal toxicity and other effects on the fetus. For most substances, fetal body weight reduction occurs in a single exposure at higher dosages than in repeated exposures but for some substances no difference is observed between the NOAELs / LOAELs of single and repeated studies.
- Malformations and variants are – by default – relevant endpoints for setting limits for acute exposures, unless information is available to indicate otherwise. For some substances no difference in NOAELs /LOAELs is observed between single and repeated exposures but for others clear differences indeed occur (probably related to characteristics of the substances such as kinetics, metabolism, mechanism of action). Whether substances could be categorized based on kinetics and mechanism of action should be further investigated.
- Skeletal variants (fused ribs, fused sternebrae, fused vertebrae/arches, way ribs) are – by default – relevant endpoints for setting of limits for acute exposures; unless information is available to conclude otherwise. The analysis shows a similar picture as for malformations and variants in general (for some substances no difference, for others a clear difference). In addition, some skeletal variants occur spontaneously and are often considered to be related to maternal toxicity. Therefore, the relevance of these effects has to be evaluated carefully.

Taken all information together two general conclusions can be formulated:

1. The critical window of sensitivity for developmental effects, including malformations, is probably somewhat larger than expected in most cases. The critical window should be viewed as a period with relatively increased risk and spans (depending on the type of effect and the type of substance) probably more than one day (see figure 1). Exposure

outside the critical window does not automatically means that there is no risk. Starting from this point of view, repeated exposure is likely to induce a larger effect compared to a single exposure at the same dose rate.

2. Using a NOAEL from a standard guideline-based (repeated) developmental toxicity study represents for most substances a conservative estimation of the NOAEL for single exposures, regardless of the endpoint under consideration. This means that also for malformations, the NOAEL from a standard study is in the majority of cases quite conservative. This should be taken into account when selecting endpoints and safety factors for acute exposure limits.

References

Ali, M.M., Murthy, R.C., Chandra, S.V. (1986) Developmental and long term neurobehavioral toxicity of low level in-utero cadmium exposure in rats. Neurobehav. Toxicol. Teratol. 8; 463-468.

Aliverti, V., Bonamoni, L., Giavini, E., Leone, V.G., Mariani, L. (1979) The extent of fetal ossification as an index of delayed development in teratogenic studies on the rat. Teratol. 20; 237-242.

Baranski, B. (1985) Effect of exposure of pregnant rats to cadmium on prenatal and postnatal development of the young. J. Hyg. Epidemiol. Microbiol. Immunol. 29; 253-262.

Barr, M. (1973) The teratogenicity of cadmium chloride in two stocks of Wistar Rats. Teratol. 7; 237-242.

Beekhuijzen, M.E.W., Verhoef, A., Klaassen, R., Rompelberg, C.J.M., Piersma, A.H. (2000) Comparison of in vitro and in vivo developmental toxicity and pharmacokinetics of phenytoin in the rat. Pharmacol. Toxicol. 87; 96-102.

Billington, R., Carmichael, N. (2000) Setting of acute reference doses for pesticides based on existing regulatory requirements and regulatory test guidelines. Food. Add. Contam. 17; 621-626.

Bolon, B., Dorman, D.C., Janszen, D., Morgan, K.T., Welsch, F. (1993) Phase-specific developmental toxicity in mice following maternal methanol inhalation. Fund. Appl. Toxicol. 21; 508-516.

Capariccio, B., Michel, R., Tournamille, J., Sentein, P. (1981) Effet du propham et du chloropropham sur le developpement embryonnaire de quelques vertebres. C.R. Soc. Biol. 175; 811-820.

Clarke, D.O., Duignan, J.M., Welsch, F. (1992) 2-Methoxyacetic acid dosimetry-Teratogenicity relationships in CD-1 mice exposed to 2-methoxyethanol. Toxicol. Appl. Pharmacol. 114; 77-87.

Collins, M.D., Tzimas, G., Hummler, H., Burgin, H., Nau, H. (1994) Comparative teratology and transplacental pharmacokinetics of all-trans-retinoic acid, 13-cis retinoic acid, and retinyl plamitate follwing daily adminitrations in rats. Toxicol. Appl. Pharmacol. 127; 132-144.

Colomina, M.T., Domingo, J.L., Llobet, J.M., Corbella, J. (1996) Effect of day of exposure on the developmental toxicity of manganese in mice. Vet. Hum. Toxicol. 38; 7-9.

Connelly, L.E., Rogers, J.M. (1994) Methanol causes posteriorization of cervical vertebrae in the mouse fetus. Teratol. 49; 393. (P31 Abstract only).

Cummings, A.M., Ebron-McCoy, M.T., Rogers, J.M., Barbee, B.D., Harris, S.T. (1991) Exposure to carbendazim during early pregnancy produces embryolethality and developmental defects. Biol. Reprod. 44 (suppl 1), 131.

Cummings, A.M., Harris, S.T., Rehnberg, G.L. (1990) Effects of mehtyl benzimidazolcarbamate during eraly pregnancy in the rat. Fund. Appl. Toxicol. 15; 528-535.

Danielsson, B., Sköld, A.C., Azarbayjani, F., Öhman, I., Webster, W. (2000) Pharmacokinetic data support pharmacologically induced embryonic dysrhythmia as explanation for fetal hydantoin syndrome in rats. Toxicol. Appl. Pharmacol. 163; 164-175.

De-Carvalho, R.R., Delgado, I.F., Souza, C.A.M., Chahoud, I., Paumgartten, F.J.R. (1994) Embryotoxicity of methanol in well-nourished and malnourished rats. Brazilian J. Med. Biol. Res. 27; 2915-2923.

Dewhurst, I.C. (2000) The use and limitations of current standard toxicological data packages in the setting of acute reference doses. Food. Add. Contam. 17; 611-615.

Ellis, W.G., De Roos, F., Kavlock, R.J., Zeman, F.J. (1988) Relationship of periventricular overgrowth to hydrocephalus in brains of fetal rats exposed to benomyl. Teratog. Carc. Mutag. 8; 377-391.

Ema, M., Amano, H., Itami, T., Kawasaki, H. (1993b) Teratogenic evaluation of di-n-butyl phtalate in rats. Toxicol. Lett. 69; 197-203.

Ema, M., Amano, H., Ogawa, Y. (1994) Characterisation of the developmental toxicity of di-n-butyl phtalate in rats. Toxicol. 86; 163-174.

Ema, M., Harazono, A., Miyawaki, E., Ogawa, Y. (1997) Effect of the day of administration on the developmental toxicity of tributyltin chloride in rats. Arch. Environ. Contam. Toxicol. 33; 90-96.

Ema, M., Harazono, A., Miyawaki, E., Ogawa, Y. (1997a) Embryolethality following maternal exposure to dibutyl phtalate during early pregnancy in rats. Bull. Environ. Contam. Toxicol. 58; 636-643.

Ema, M., Harazono, A., Miyawaki, E., Ogawa, Y. (1997b) Developmental effects of di-n-butyl phthalate after a single administration in rats. J. Appl. Toxicol. 17(4): 223-229.

Ema, M., Itami, T., Kawasaki, H. (1991a) Teratogenicity of butyl benzyl phtalate in ratsJapanase teratology society abstracts 44(6); 16B.

Ema, M., Itami, T., Kawasaki, H. (1991b) Evaluation opf the embryolethality of butyl benzyl phtalate by conventional and pair-feeding studies in rats. J. Appl. Toxicol. 11; 39-42.

Ema, M., Itami, T., Kawasaki, H. (1992a) Embryolethality and teratogenicity of butyl benzyl phtalate in rats. J. Appl. Toxicol. 12; 179-183.

Ema, M., Itami, T., Kawasaki, H. (1992b) Effect of dosing period of exposure on the developmental toxicity of butyl benzyl phtalate in rats. J. Appl. Toxicol. 12; 57-61.

Ema, M., Itami, T., Kawasaki, H. (1992c) Teratogenic evaluation of butyl benzyl phtalate in rats by gastric intubation. Toxicol. Lett. 61; 1-7.

Ema, M., Itami,. T., Kawasaki, H. (1993) Teratogenic phase specificity of butyl benzyl phtalate in rats. Toxicol. 79; 11-19.

Ema, M., Kurosaka, R., Amano, H., Ogawa, Y. (1994a) Embryolethality of butyl benzyl phtalate during early pregnancy in rats. Teratol. 50(6); 17B.

Ema, M., Kurosaka, R., Amano, H., Ogawa, Y. (1994b) Embryolethality of butyl benzyl phtalate during early pregnancy in rats. Reprod. Toxicol. 8; 231-236.

Ema, M., Kurosaka, R., Amano, H., Ogawa, Y. (1995) Comparative developmental toxicity of n-butyl benzyl phtalate and di-n-butyl phtalate in rats. Arch. Environ. Contam. Toxicol. 28; 223-228.

Ema, M., Miyawaki, E., Harazono, A., Kawashima, K., Ogawa, Y. (1998) Developmental toxicity of dibutyl phtalate after a single administration in rats. J. Toxicol. Sci. 23 (suppl. II); 313.

Ema, M., Miyawaki, E., Kawasaki, K. (1998) Reproductive effects of butyl benzyl phtalate in pregnant and pseudopregnant rats. Reprod. Toxicol. 12; 127-132.

Ema, M., Miyawaki, E., Kawashima, K. (1998) Further evaluation of developmental toxicity of di-n-butyl phtalate follwoing adminstration during late pregnancy in rats. Toxicol. Lett 98; 87-93.

Ema, M., Miyawaki, E., Kawashima, K. (1999) Developmental effects of plasticizer butyl benzyl phtalate after a single adminstration in rats. J. Appl. Toxicol. 19; 357-365.

Ema, M., Miyawaki, E., Kawashima, K. (1999) Developmental toxicity of triphenyltin chloride after administration on three consequetive days during organogenesis in rats. Bull. Enviorn. Contam. Toxicol. 62; 363-370.

Ema, M., Miyawaki, E., Kawashima, K. (2000a) Critical period for adverse effects on development of reproductive system in male offsrping of rats given di-n-butyl phtalate during late pregnancy. Toxicol. Lett. 111; 271-278.

Ema, M., Miyawaki, E., Kwashima, K. (2000b) Effects of dibutyl phtalate on reproductive function in pregnant and pseudopregnant rats. Reprod. Toxicol. 14; 13-19.

Ema, M., Murai, T., Itami, T., Kawasaki, H. (1990a) Embryotoxicity of plasticizer butyl benzyl phtalate in rats. Teratol. 42; 42A.

Ema, M., Murai, T., Itami, T., Kawasaki, H. (1990b) Evaluation of the Teratogenic potential of the plasticizer butyl benzyl phtalate in rats. J. Appl. Toxicol. 10; 339-343.

Fueston, M.H., Kerstetter, S.L., Wilson, P.D. (1990) Teratogenicity of 2-methoxyethanol applied as a single dermal dose to rats. Fund. Appl. Toxicol. 15; 448-456.

Hazelhoff Roelfzema, W. (1988) Effects of long term cadmium exposure on rat placenta and consequences for the offspring. Ph.D. Thesis, University of Amsterdam, The Netherlands.

Holson, J.F., Stump, D.G., Clevidence, K.J., Knapp, J.F., Farr, C.H. (2000) Evaluation of the prenatal developmental toxicity of orally administered arsenic trioxide in rats. Food Chem. Toxicol. 38; 459-466.

Horton, V.L., Sleet, R.B., John-Green, J.A., Welsch, F. (1985) Developmental phase-specific and dose-related teratogenic effects of ethylene glycol monomethyl ehter in CD-1 mice. Toxicol. Appl. Pharmacol. 80; 108-118.

Hovland, D.N., Machado, A.F., Scott, W.J., Collins, M.D. (1999) Differential sensitivity of the SWV and C57BL/6 mouse strains to the teratogenic action of single administrations of cadmium given throughout the period of anterior neuropore closure. Teratol. 60; 13-21.

Hung, C.F., Lin, K.R., Lee, C.S. (1986) Experimental production of congenital malformations of the central nervous system in rat fetuses by single dose intragastric administration of ethylenethiourea. Proc. Natl. Sci. Counc. B. ROC; 10; 127-136.

Huuskonen, H., Unkila, M., Pohjanvirta, R., Tuomisto, J. (1994) Developmental toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the most TCDD-resistant and –susceptible rat strains. Toxicol. Appl. Pharmacol. 124; 174-180.

Itami, T., Ema, M., Kawasaki, H. (1990) Increased placental weight induced by tributyltin chloride in rats. Teratol. 42; 42A.

Janardhan, A., Sattur, P.B., Sisodia, P. (1984) teratogenicity of methyl benzimidazole carbamate in rats and rabbits. Bull. Environ. Contam. Toxicol. 33; 257-263.

Kavlock, R.J., Chernoff, N., Gray, L.E., Gray, J.A., Whitehouse, D. (1982) teratogenic effects of benomyl in the Wistar rat and CD-1 mouse with emphasis on the route of administration. Toxicol. Appl. Pharmacol. 62; 44-54.

Kavlock, R.J., Chernoff, N., Rogers, E.H. (1985) The effects of acute maternal toxicity on fetal development in the mouse. Teratog. Carcinog. Mutagen. 5; 3-13.

Khera, K.S. (1984) Maternal Toxicity – A possiblde factor in fetal malformations in mice. Teratol. 29; 411-416.

Khera, KS. (1985) Maternal Toxicity – A Possible etiological factor in embryo-fetal deaths and fetal malformations of rodent-rabbit species. Teratol. 31: 129-153

Khera, K.S., Whalen, C., Iverson, F. (1983) Effects of pretreatment with SKF-525A, N-methyl-2-thioimidazole, sodium phenobarbital, or 3-methylcholanthrene on ethylenethiourea-induced teratogenicity in hamsters. J. Toxicol. Environ. Health 11; 287-300.

Lau, C., Cameron, A.M., Rogers, J.M., Shuey, D.L., Kavlock, R.J. (1992) Development of biologically based dose-response models: Correlation between developmental toxicity of 5-fluorouracil and its inhibition of thymidylate synthetase activity in the rat embryo. Teratol. 45; 457. (no. 17, Abstract only).

Levine, B.S., Youssef, A., Kirchner, D.L., Mercieca, M.D. (1999) Retinyl palmitate as a positive control agent in rat and rabbit developmental studies. Toxicol. Methods 9; 229-243.

Maechemer, L., Lorke, D. (1981) Embryotoxic effect of cadmium on rats upon oral administration. Toxicol. Appl. Pharmacol. 58; 438-443.

Marks, T.A., Black, D.L., Terry, R.D., Branstetter, D.G., Kirton, K.T. (1990) Varaibility in the developmental toxicity of bropirimine with the day of administration. Teratol. 42; 55-66.

Marks, T.A., Poppe, S.M. (1988) Developmental toxicity of bropirimine in rats after oral administration. Teratol. 38; 7-14.

Mas, A., Holt, D., Webb, M. (1988) The acute toxicity and teratogenicity of nickel in pregnant rats. Toxicol. 35; 47-57.

Minta, M., Biernacki, B. (1982) Embryotoxicity of carbendazim in hamsters, rats, and rabbits. Bull. Vet. Inst. Putawy 25; 42-52.

Nagano, K., Nakayama, E., Oobayashi, H., Yamada, T., Adachi, H., Nishizawa, T., Ozawa, H., Nakaichi, M., Okuda, H., Minami, K., Yamazaki, K. (1981) Embryotoxic effects of ethylene glycol monomethyl ether in mice. Toxicol. 20; 335-343.

Nakatsuka, T., Matsumoto, H., Ikemoto, F. (1995) Thiabendazole – oral developmental toxicity study in mice. Unpublished report no. TT #94-9818, Banyu Pharmaceuticals Co., Ltd., Japan. Summarised in background paper by M.E.J. Pronk & J.S. Schefferlie (RIVM, NL) for JECFA.

Narotsky, M.G., Best, D.S., Guidici, D.L., Bielmeier, S.R. (1999) Trichloroethylene (TCE)-iduced pregnancy loss in F344 rats and partial rescue by chorionic gonadotropin (hCG). Teratol. 59; 402. (P16 Abstract only).

Narotsky, M.G., Brownie, C.F., Kavlock, R.J. (1997a) Critical period of carbon tetrachloride-induced pregnancy loss in Fischer-344 rats, with insights into the detection of resorption sites by ammonium sulfide staining. Teratol. 56; 252-261.

Narotsky, M.G., Hamby, B.T., Best, D.S., Kavlock, R.J. (1995) Carbon tetrachloride (CCl4)-induced pregnancy loss in F-344 rats: lutheinizing hormone (LH) levels and rescue by human chorionic gonadotropin (hCG). Biol. Reprod. 52 (suppl. 1); 172 (Abstract no. 462).

Narotsky, M.G., Hamby, B.T., Mitchell, D.S., Kavlock, R.J. (1993) Evaluation of the critical period of bromodichloromethane-induced full-litter resorption in F-344 rats. Teratol. 47; 429. (P58 Abstract only).

Narotsky, M.G., Pegram, R.A., Kavlock, R.J. (1997b) Effects of dosing vehicle on developmental toxicity of bromodichloromethane and carbon tetrachloride in rats. Fund. Appl. Toxicol. 40; 30-36.

Narotsky, M.G., Weller, E.A., Chinchilli, V.M., Kavlock, R.J. (1995) Nonadditive developmental toxicity in mixtures of trichlorethylene, di(2-ethylhexyl) phtalate, and heptachlor in a 5x5x5 design. Fund. Appl. Toxicol. 27; 203-216.

Nau, H. (1990) Correlation of transplacental and maternal pharmacokinetics of retinoids during organogenesis with teratogenicity. Methods Enzymol., 190, 437-448.

Nelson, B.K., Brightwell, W.S., MacKenzie, D.R., Khan, A., Burg, J.R., Weigel, W.W., Goad, P.T. (1985) Teratological assessment of methanol and ethanol at high inhalation levels in rats. Fund. Appl. Toxicol. 5; 727-736.

Nelson, B.K., Brigthwell, W.S., Krieg, E.F. (1990) Developmental toxicity of industrial alcohols: a summary of 13 alcohols administrated by inhalation to rats. Toxicol. Ind. Health 6; 373-387.

Nelson, B.K., Conover, D.L., Shaw, P.B., Werren, D.M., Edwards, R.M., Hoberman, A.M. (1994) Interactive developmental toxicity of radiofrequnecy radiation and 2-methoxyethanol in rats. Teratol. 50; 275-293.

Nemec, M. Holson, J., Naas, D., Oberholtzer, K., Varsho, B., MCallister, D. (1992b) The single day exposure inhalation developmental toxicity studies of FM-100^{TM} in the rat. Teratol. 45; 501. (no.158 Abstract only).

Nemec, M., Holson, J., Naas, D., Knapp, J., Lamb, I., MCallister, D. (1992a) The developmental toxicity of FM-100^{TM} in the rat and rabbit following repeated exposure by inhalation. Teratol. 45; 475-476. (no. 77 Abstract only).

NRC (2001) Standing operating procedures for developing acute exposure guideline levels for hazardous chemicals. National Research Council, Committee on Toxicology, Subcommittee on Acute Exposure Guideline Levels, USA. National Academy Press, Washington D.C.

Ogata, A., Ando, H., Kubo, Y., Hiraga, K. (1984) Teratogenicity of thiabendazole in ICD mice. Food Chem. Toxicol. 22; 509-520.

Ogata, A., Fujitani, T., Yneyama, M., Sasaki, M. (1989) Glutathione and cysteine enhance and diethylmaleate reduces thiabendazole teratogenicity in mice. Food Chem. Toxicol. 27; 117-123.

Ogata, A., Yoneyama, M., Sasaki, M., Suzuki, K., Imamichi, T. (1987) Effects of pretreatment with SKF-525A or sodium phenobarbital on thiabendazole-induced teratogenicity in ICR mice. Food Chem. Toxicol. 25; 119-124.

Olling, M., Piersma, A.H., Bruil, M.A., Verhoef, A., Klaassen, R. (1995) Toxicokinetiek en embryotoxische effecten van vitamine A na eenmalige orale toediening van verschillende doses retinylpalmitate aan drachtige ratten. RIVM report no. 642810 002, dd. October 1995. National Institute of Public Health and Environment, Bilthoven, The Netherlands. (In Dutch).

ORTEP (1987) Toxicology and analytics of the tributyltins – the present status. Workshop proceedings of May 15-16th 1986, Berling. Published by Organotin Environmental Programma (ORTEP-Association), The Netherlands.

Padmanabhan, R. (1986) The effect of cadmium on placental structure and its relation to fetal malformations in the mouse. Z. Mikrisk.-anat. Forsch. 100; 419-427.

Padmanabhan, R., Hameed, M.S. (1990) Characteristics of the limb malformations induced by maternal exposure to cadmium in the mouse. Reproductive Toxicol. 4; 291-304.

Piersma, A.H., Bode, W., Verhoef, A., Olling, M. (1996) Teratogenicity of a single oral dose of retinyl palmitate in the rat, and the role of dietary vitamin A status. Pharmacol. Toxicol. 79; 131-135.

Piersma, A.H., Verhoef, A., Dortant, P.M. (1995) Evaluation of the OECD 421 repoductive toxicity screening test protocol using butyl benzyl phtalate. Toxicol. 99; 191-197.

Piersma, A.H., Verhoef, A., Olling, M. (1996a) A single oral dose of retinylpalmitate may cause a transient delay in neural tube closure in rat embryos. Teratol. 53; 120. (P65 Abstract only).

Piersma, A.H., Verhoef, A., Olling, M. (1996b) Teratogenicity of a single oral dose of retinyl palmitate in the rat, and the role of dietary vitamin A status. Pharmacol. Toxicol. 79; 131-135.

Piersma, A.H., Verhoef, A., Te Biesebeek, J., Pieters, M.N., Slob, W. (2000) Developmental toxicity of butyl benzyl phtalate in the rat using a multiple dose study design. Reprod. Toxicol. 14; 417-425.

Piersma, A.H. et al. (2002) Evaluation of the TTC concept when considering teratogenic effects. RIVM report in preparation.

Pronk, M.E.J., Schefferlie, G.J. (2001) Thiabendazole – background paper. Evaluation of studies provided for the setting of an ARfD. Unpublished evaluation by RIVM/CSR for WHO-JECFA.

Ritchie, H.E., Webster, W.S., Eckhoff, C., Oakes, D.J. (1998) Model predicting the teratogenic potential of retinyl palmitate, using a combined in vivo/in vitro approach. Teratol. 58; 113-123.

Rogers, J.M. Mole, M.L., Chernoff, N., Barbee, B.D., Turner, C.I., Logsdon, T.R., Kavlock, R.J. (1993) The developmental toxicity of inhaled methanol in the CD-1 mouse, with quantitative dose-response modeling for estimation of benchmark doses. Teratol. 47; 175-188.

Rogers, J.M., Barbee, B.D., Mole, M.L. (1995) Exposure concentration and time (CxT) relationships in the developmental toxicity of methanol in mice. Toxicologist 15; 164.

Rogers, J.M., Chernoff, N., Mole, M.L. (1991) Developmental toxicity of inhaled methanol in mice. Toxicologist 11; 344.

Rogers, J.M., Mole, M.L. (1997) Critical periods of sensitivity to the developmental toxicity of inhaled methanol in the CD-1 mouse. Teratol. 55; 364-372.

Rogers, J.M., Setzer, R.W., Shuey, D.L., Narotsky, M.G., Lau, C., Rogers, E., Abbott, B.D., Copeland, M.F., Logsdon, T., Kavlock, R.J. (1992)) Development of biologically based dose-response models: 5-fluorouracil dose response based on fetal outcome and linkage to mechanistic endpoints. Teratol. 45; 457. (no. 18, Abstract only).

Rowland, J.R., Binkerd, P.E., Hendrickx, A.G. (1990) Developmental toxicity and pharmacokenetics of oral and intravenous phenytoin in the rat. Reprod. Toxicol. 4; 191-202.

Ruijten, M., Van Doorn, R., Habets, T., Cenin, Th., Van Haagen, R. (2000) Interventiewaarden gevaarlijke stoffen no. 8. Ministerie van Volkshuisvesting, Ruimtelijke ordening en milieubeheer / Ministerie van Binnenlandse zaken en Koningrijksrelaties.

Ryan, B.M., Hatoum, N.S., Mallett, E.J., Yermakoff, J.K. (1994) A pilot developmental toxicity study of methanol in folate-deficiënt Long Evans rats. Teratol. 49; 399 (P54 Abstract only).

Saillenfait, A.M., Payan, J.P., Fabry, J.P., Beydon, D., Langonne, I., Gallisot, F., Sabate, J.P. (1998) Assessment of the developmental toxicity, metabolism, and placental transfer of di-n-butyl phtalate adminstered to pregnant rats. Toxicol. Sci. 45; 212-224.

Sánchez, D.J., Domingo, J.L., Llobet, J.M., Keen, C.L. (1993) Maternal and developmental toxicity of manganese in the mouse. Toxicol. Lett. 69; 45-52.

Sato, F., Watanabe, T., Hoshi, E., Endo, A. (1985) Teratogenic effect of maternal zinc deficiency and its co-teratogenic effect with cadmium. Teratol. 31; 13-18.

Saxena, D.K., Murthy, R.C., Chandra, S.V. (1986) Embryotoxic and teratogenic effects of interaction of cadmium and lindane in rats. Acta Pharmacol. Toxicol. 59; 175-178.

Sitarek, K. (2001) Embryolethality and teratogenic effects of carbendazim in rats. Teratog. Carcinog. Mutagen. 21; 335-340.

Sleet, R.B. (1994) Serine attenuation of 2-methoxyethanol (ME) induced limb dysmorphogenesis in the rat: assessment of serine's action following its administration with and after ME treatment. Teratol. 49; 416. (P120 Abstract only).

Staples, R.E., 1980. Benomyl: Teratogenicity in the rat after administration by gavage. DPR Vol. 294-065, #36320. In WHO-JMPR evaluations 1995 part II (Toxicological and Environmental); Benomyl pp. 3-31.

Staples, R.E., 1982. Benomyl gavage: Teratogenicity in the rat; DPR Vol. 294-065, #36323. In WHO-JMPR evaluations 1995 part II (Toxicological and Environmental); Benomyl pp. 3-31.

Stump, D.G., Holson, J.F., Fleeman, T.L., Nemec, M.D., Farr, C.H. (1999) Comparative effects of single intraperitoneal or oral doses of sodium aresenate or arsenic trioxide during in utero development. Teratol. 60; 283-291.

Sunderman, F.W., Allpass, P.R., Mitchell, J.M., Baselt, R.C. (1979) Eye malformations: induction by prenatal exposure to nickel carbonyl. Science 203; 550-553.

Sunderman, F.W., Shen, S.K., Mitchell, J.M., Allpass, P.R., Damjanov, I. (1978) Embryotoxicity and fetal toxicity of nickel in rats. Toxicol. Appl. Pharmacol. 43; 381-390.

Sunderman, F.W., Shen, S.K., Reid, M.C., Allpass, P.R. (1980) Teratogenicity and embryotoxicity of nickel carbonyl in syrian hamsters. Teratog. Carc. Mutag. 1; 223-233.

Tanaka, T. (1997a) Reproductive and neurobehavioural effects of chlorpropham administered to mice in the diet. Toxicol. Ind. Health 13; 715-726.

Tanaka, T., Fujitani, T., Takahashi, O., Oishi, S., Yoneyama, M. (1997b) Develpmental toxicity of chlorpropham in mice. Reprod. Toxicol. 11; 697-701.

Teramoto, S., Shingu, A., Kaneda, M., Saito, R. (1978) Teratogenicity studies with ethylenethiourea in rats, mice and hamsters. Cong. Anom. 18; 11-17.

Terry, K., Eslwick, B.A., Stedman, D.B., Welsch, F. (1994) Developmental phase alters dosimetry-teratogenicity relationship for 2-methoxyethanol in CD-1 mice. Teratol. 49; 218-227.

Toraason, M., Stringer, B., Stober, P., Hardin, B.D. (1985) Electrocardiographic study of rat fetuses exposed to ethylene glycol monomethyl ether (EGME). Teratol. 32; 33-39.

Van Raaij (2001) Guidance document for setting an Acute Reference Dose in Dutch National Pesticide evaluations. RIVM report no. 620555 002, may 2001, National Institute of Public Health and Environment, Bilthoven, The Netherlands.

Vergieva, T. (1998) Singel day treatment – feasible tool in revealing not dependent on maternal toxicity teratogenic potential. Reprod. Toxicol. 444; 191-199.

Webster, W.S. (1978) Cadmium-induced fetal growth retardation in the mouse. Arch. Environ. Health 33; 36-42.

Welsch, F., Terry, K.K., Stedman, D.B., Elswick, B.A. (1996) Linking embryo dosimetry and teratogenic response to 2-methoxyethanol at different stages of gestation in mice. Occup. Hyg. 2; 121-130.

Wise, L.D. (1990) Thiabendazole – oral developmental toxicity study in rats. Unpublished report no. TT #90-713-0, Merck Sharpe & Dohme Research Laboratories, USA. Summarised in background document by M.E.J. Pronk & J.S. Schefferlie (RIVM, NL) for JECFA.

Youssef, A.F. (1991) Teratogenicity of methanol by the oral route. Toxicologist 11; 344.

APPENDIX A: Selected NOAEL and LOAEL values for the endpoints Maternal Toxicity, Resorptions, Fetal body weight, and Number of Fetuses with malformations/variants

NOAEL and LOAEL values are expressed as mg/kg bw/day (oral, dermal) or as ppm (inhalation)

Overview of results: Relevance of developmental effects for acute exposure
Type of endpoint: **Maternal Toxicity**

			Absolute values in (mg/kg bw or ppm)						Percentage of repeated				NLR
Route	Species	Substance	NOAEL repeated	LOAEL repeated	NOAEL 2-3 days	LOAEL 2-3 days	NOAEL single	LOAEL single	NOAEL 2-3 days	LOAEL 2-3 days	NOAEL single	LOAEL single	NOAEL single/LOAEL rep
oral-gavage	rat	Arsenic trioxide	5	10			20	30	0	0	400	300	2,00
oral-gavage	rat	Benomyl	62,5	125					0	0			
oral-gavage	rat	Bropirimine	25	50				200	0	0		400	
oral-gavage	rat	Butyl Benzyl Phtalate	375	500	600	750	1000	1500	160	150	267	300	2,00
sc	mice	Cadmium	2,8	5,6			8	10	0	0	286	179	1,43
oral-gavage	rat	Carbendazim	8	35			30	60	0	0	375	171	0,86
oral-gavage	rat	Carbon tetrachloride	25	50				150	0	0		300	
	mice	Chlorpropham	200	800			3000		0	0	1500		3,75
oral-gavage	rat	Di-n-butylphtalate	250	500	500	1000	1000	1500	200	200	400	300	2,00
oral-gavage	rat	Deltamethrin	3	6			30	38	0	0	1000	633	5,00
oral-gavage	rat	ETU	50	80			240		0	0	480		3,00
oral-gavage	hamster	ETU	810				2400		0	#DIV/0!	296		
inhalation	rat	FM-100 TM	4000	10000				20000	0	0		200	
s.c. injection	mice	Manganese	4	8					0	0			
oral-gavage	rat	Methanol	357	700			2080	4240	0	0	583	606	2,97
inhalation	mice	Methanol	5000	10000	10000	15000	10000	15000	200	150	200	150	1,00
oral-gavage	rat	2-Methoxyethanol	25	50			150		0	0	600		3,00
oral-gavage	mice	2-Methoxyethanol	125	250	250		500		200	0	400		2,00
dermal	rat	2-methoxyethanol	48	97			500	1000	0	0	1042	1031	5,15
i.m.	rat	Nickel					8	12	#DIV/0!	#DIV/0!			
oral		Paclobutrazole		50					#DIV/0!	0			
oral-gavage	rat	Phenytoin	150	375			500	1500	0	0	333	400	1,33
oral-gavage	rat	Retinyl palmitate							#DIV/0!	#DIV/0!			
oral-gavage	mice	Thiabendazole	25	100			965	1157	0	0	3860	1157	9,65
oral-gavage	rat	Tributyltin (Cl, O)	5	9				200	0	0		2222	
oral-gavage	rat	Trichloroethylene	320	475					0	0			

Overview of results: Relevance of developmental effects for acute exposure

Type of endpoint: **Embryo/foetal resorptions**

Route	Species	Substance	Absolute values						Percentage of repeated				NLR
			NOAEL repeated	LOAEL repeated	NOAEL 2-3 days	LOAEL 2-3 days	NOAEL single	LOAEL single	NOAEL 2-3 days	LOAEL 2-3 days	NOAEL single	LOAEL single	NOAEL single/LOAEL rep
oral-gavage	rat	Arsenic trioxide	10	15			20	30	0	0	200	200	1,33
oral-gavage	rat	Benomyl	62,5	125			62,5	125	0	0	100	100	0,50
oral-gavage	rat	Bropirimine	100	200			400		0	0	400		2,00
oral-gavage	rat	Butyl Benzyl Phtalate	500	750	600	750	1000	1500	120	100	200	200	1,33
sc	mice	Cadmium						4	#DIV/0!	#DIV/0!			
oral-gavage	rat	Carbendazim	20	35			60	150	0	0	300	429	1,71
oral-gavage	rat	Carbon tetrachloride	25	50				150	0	0		300	
	mice	Chlorpropham		970			1500	3000	#DIV/0!	0		309	1,55
oral-gavage	rat	Di-n-butylphtalate	500	630	750	1000	1000	1500	150	159	200	238	1,59
oral-gavage	rat	Deltamethrin	12				38		0	#DIV/0!	317		
oral-gavage	rat	ETU	40	50				60	0	0		120	
oral-gavage	hamster	ETU	270	810			1800	2400	0	0	667	296	2,22
inhalation	rat	FM-100 TM							#DIV/0!	#DIV/0!			
s.c. injection	mice	Manganese	2	4				50	0	0		1250	
oral-gavage	rat	Methanol	2500	4000			4240		0	0	170		1,06
inhalation	mice	Methanol	5000	7500	5000	10000	10000	15000	100	133	200	200	1,33
oral-gavage	rat	2-Methoxyethanol	25	50	75		100	125	300	0	400	250	2,00
oral-gavage	mice	2-Methoxyethanol	62,5	125		250	450	500	0	200	720	400	3,60
dermal	rat	2-methoxyethanol	48	97			1000	2000	0	0	2083	2062	10,31
i.m.	rat	Nickel	3	4			8	12	0	0	267	300	2,00
oral		Paclobutrazole	200				500		0	#DIV/0!	250		
oral-gavage	rat	Phenytoin	750	1125			500	1500	0	0	67	133	0,44
oral-gavage	rat	Retinyl palmitate	90			1000	100	300	0	#DIV/0!	111		
oral-gavage	mice	Thiabendazole	700	1300			1389	1667	0	0	198	128	1,07
oral-gavage	rat	Tributyltin (Cl, O)	15		3,1	6,3	100	200	21	#DIV/0!	667		
oral-gavage	rat	Trichloroethylene	320	475			960	1280	0	0	300	269	2,02

Overview of results: Relevance of developmental effects for acute exposure

Type of endpoint: **Fetal Body Weight**

Route	Species	Substance	Absolute values						Percentage of repeated				NLR
			NOAEL repeated	LOAEL repeated	NOAEL 2-3 days	LOAEL 2-3 days	NOAEL single	LOAEL single	NOAEL 2-3 days	LOAEL 2-3 days	NOAEL single	LOAEL single	NOAEL single/LOAEL rep
oral-gavage	rat	Arsenic trioxide	5	10			20	30	0	0	400	300	2,00
oral-gavage	rat	Benomyl	31,2	62,5			62,5	125	0	0	200	200	1,00
oral-gavage	rat	Bropirimine	100	200					0	0			
oral-gavage	rat	Butyl Benzyl Phtalate	185	375	600	750	1000	1500	324	200	541	400	2,67
sc	mice	Cadmium		2,8			3	4	#DIV/0!	0		143	1,07
oral-gavage	rat	Carbendazim	8	35			15	30	0	0	188	86	0,43
oral-gavage	rat	Carbon tetrachloride	75				150		0	#DIV/0!	200		
	mice	Chlorpropham	900	970			1500	3000	0	0	167	309	1,55
oral-gavage	rat	Di-n-butylphtalate	250	500		750	1000	1500	0	150	400	300	2,00
oral-gavage	rat	Deltamethrin	12				38		0	#DIV/0!	317		
oral-gavage	rat	ETU	20	30					0	0			
oral-gavage	hamster	ETU	90	270			600	1200	0	0	667	444	2,22
inhalation	rat	FM-100 TM	4000	10000			40000		0	0	1000		4,00
s.c. injection	mice	Manganese	4	8				50	0	0		625	
oral-gavage	rat	Methanol		2500				1040	#DIV/0!	0		42	
inhalation	mice	Methanol	7500	10000	10000	15000	5000	10000	133	150	67	100	0,50
oral-gavage	rat	2-Methoxyethanol	25	50	75		125	150	300	0	500	300	2,50
oral-gavage	mice	2-Methoxyethanol	62,5	125		250	175	250	0	200	280	200	1,40
dermal	rat	2-methoxyethanol		48			500	1000	#DIV/0!	0		2083	10,42
i.m.	rat	Nickel	4				12	16	0	#DIV/0!	300		
oral-gavage	rat	Phenytoin	150	200			500	1000	0	0	333	500	2,50
oral		Paclobutrazole	200				500		0	#DIV/0!	250		
oral-gavage	rat	Retinyl palmitate	90			1000	330	1000	0	#DIV/0!	367		
oral-gavage	mice	Thiabendazole	25	100			30	60	0	0	120	60	0,30
oral-gavage	rat	Tributyltin (Cl, O)		5	9,4	12,5		100	#DIV/0!	250		2000	
oral-gavage	rat	Trichloroethylene	844	1125					0	0			
											350	476	2,30
											223	611	2,46

Overview of results: Relevance of developmental effects for acute exposure

Type of endpoint: **Number of fetuses with malformations or variants**

Route	Species	Substance	Absolute values						Percentage of repeated				NLR
			NOAEL repeated	LOAEL repeated	NOAEL 2-3 days	LOAEL 2-3 days	NOAEL single	LOAEL single	NOAEL 2-3 days	LOAEL 2-3 days	NOAEL single	LOAEL single	NOAEL single/LOAEL rep
oral-gavage	rat	Arsenic trioxide	10				30		0	#DIV/0!	300		
oral-gavage	rat	Benomyl	31,2	62,5			31,2	62,5	0	0	100	100	0,50
oral-gavage	rat	Bropirimine	25	50					0	0			
oral-gavage	rat	Butyl Benzyl Phtalate	500	750	600	750	1000	1500	120	100	200	200	1,33
sc	mice	Cadmium						4	#DIV/0!	#DIV/0!			
oral-gavage	rat	Carbendazim	8	35			150		0	0	1875		4,29
oral-gavage	rat	Carbon tetrachloride							#DIV/0!	#DIV/0!			
	mice	Chlorpropham	970				750	1500	0	#DIV/0!	77		
oral-gavage	rat	Di-n-butylphtalate	500	661	630	750	500	1000	126	113	100	151	0,76
oral-gavage	rat	Deltamethrin	6	12			38		0	0	633		3,17
oral-gavage	rat	ETU	10	20				60	0	0		300	
oral-gavage	hamster	ETU	90	270			600	1200	0	0	667	444	2,22
inhalation	rat	FM-100 TM	4000	10000			20000	30000	0	0	500	300	2,00
s.c. injection	mice	Manganese	8	16				50	0	0		313	
oral-gavage	rat	Methanol	350	700			1040	2080	0	0	297	297	1,49
inhalation	mice	Methanol	1000	2000	5000	10000	2000	5000	500	500	200	250	1,00
oral-gavage	rat	2-Methoxyethanol		25			125	150	#DIV/0!	0		600	5,00
oral-gavage	mice	2-Methoxyethanol	31,2	62,5			100	175	0	0	321	280	1,60
dermal	rat	2-methoxyethanol		48			250	500	#DIV/0!	0		1042	5,21
i.m.	rat	Nickel	4				16		0	#DIV/0!	400		
oral-gavage	rat	Phenytoin	375	750			150	500	0	0	40	67	0,20
oral		Paclobutrazole	50	200				200	0	0		100	
oral-gavage	rat	Retinyl palmitate		30		1000	165	300	#DIV/0!	3333		1000	5,50
oral-gavage	mice	Thiabendazole	25	100			129	240	0	0	516	240	1,29
oral-gavage	rat	Tributyltin (Cl, O)	15		3,1	6,3	100	200	21	#DIV/0!	667		
oral-gavage	rat	Trichloroethylene	475	633					0	0			
											430,8	355,2	2,37
											439,8	291,6	1,80

APPENDIX B: Primary data for individual studies

Tables are arranged by substance name in alphabetical order

Arsenic trioxide – oral – rats

Arsenic trioxide (oral, gavage) at 0, 1, 2.5, 5, 10, or 15 mg/kg bw/day during 14 days preconception to GD 19 in rats (Holson et al., 2000).
Arsenic trioxide (oral, gavage) at doses of 0, 5 [3.8], 10 [7.6], 20 [15.2], 30 [22.7] mg/kg bw/d [As] at GD 9 in rats (Stump et al., 1999).

Total treatment days	33	1
GD	Prenatal day –14 till GD 19	9
Corpora Lutea	15/??	
# implantations / dam	15/??	
Pre-implantation loss	15/??	
Embryonal / foetal resorptions	10/15	20/30
Post-implantation loss	10/15	20/30
Foetal / Litter size		
Number of live fetuses	10/15	20/30
Foetal death	10/15	
Sex ratio	10/??	20/30
Body weights (foet/ litter)	5/10	20/30
Malformations / Variants	10/??	30/??
- external	10/??	
- visceral	10/??	
- skeletal delayed ossification	5/10	
Maternal toxicity	5/10	20/30
	Holson et al., 2000	Stump et al., 1999

Benomyl – oral - rats

Benomyl (oral gavage) at 15.6, 62.5, 125, 500 or 1000 mg/kg bw/day during GD 6-15 or GD 7, 9, 11, or 13 (Vergeiva, 1998)
Benomyl (oral gavage) at 62.5 mg/kg bw at GD 7-16 or 7-20 in SD rats (Ellis et al., 1988)
Benomyl (oral, gavage) with 0, 3, 6.25, 10, 20, 30, 62.5, 125 mg/kg bw/d at GD 7-16 (Staples, 1980 & Staples, 1982)
Benomyl (oral gavage) at 0, 15.6, 31.2, 62.5, 125 mg/kg bw during GD 7-15 in rats (Kavlock et al., 1982)

Total treatment days	10	10 / 14	9	10	10	1	1	1	1
Day(s) of treatment	6-15 gestation	GD 7-16/20	7-15	GD 7-16	GD 7-16	7 gestation	9 gestation	11 gestation	13 gestation
Corpora lutea	62.5/??			125/??		1000/??	500/??	1000/??	1000/??
Implantations per dam		62.5/??	125/??	125/??					
Pre-implantation losses									
Embryonal/foetal resorptions	62.5/??	??/62.5		62.5/125	62.5/125	1000/??	62.5/125	62.5/125	1000/??
Post-implantation losses									
Foetusses/litter size	15.6/62.5					1000/??	62.5/125	62.5/125	1000/??
No of viable fetuses	15.6/62.5					1000/??	62.5/125	62.5/125	1000/??
Dead foetusses	15.6/62.5		62.5/125	62.5/125	65.5/125	1000/??		62.5/125	1000/??
Sex ratio				125/??					
Body weight	62.5/??		31.2/62.5	30/62.5	31.2/62.5	1000/??	62.5/125	62.5/125	62.5/125
Abnormal foetuses	15.6/62.5	??/62.5	31.2/62.5	30/62.5	31.2/62.5	500/1000	500/??	62.5/125	15.6/62.5
Malformations - External CNS effects - Visceral hydronephrosis - Skeletal Delayed ossification Supernumary ribs Fused ribs/ vertebrae			 31.2/62.5 62.5/125 31.2/62.5	 30/62.5 62.5/125	 31.2/62.5 31.2/62.5				
Maternal toxicity	62.5/??	62.5/??	62.5/125	125/??	62.5/125				
	Vergeiva 1998	Ellis et al., 1988	Kavlock et al., 1982	Staples, 1980; Staples, 1982	Kavlock et al., 1982	Vergeiva, 1998	Vergeiva, 1998	Vergeiva, 1998	Vergeiva, 1998

Benomyl – oral (diet) - rats

Benomyl (oral, diet) with 0, 8.6, 43.5, 209.5 and 372.9 mg/kg bw/d at GD 6-15 in rats (Sherman et al., 1975)
Benomyl (oral, diet) with 0, 169, 298, 505 mg/kg bw/d at GD 7-16 (Kavlock et al., 1982)

Total treatment days	10	10				
Day(s) of treatment	GD 6-15	GD 7-16				
Corpora lutea						
Implantations per dam	372/??					
Pre-implantation losses	372/??					
Embryonal/foetal resorptions	372/??					
Post-implantation losses						
Foetusses/litter size						
No of viable fetuses	372/??					
Dead foetusses	372/??					
Sex ratio						
Body weight	372/??	169/298				
Abnormal foetuses	209/372					
Malformations - External CNS effects - Visceral hydronephrosis - Skeletal Delayed ossification Fused ribs	 209/372 209/372	 169/298 298/505				
Maternal toxicity	372/??	169/298				
	Sherman et al., 1975	Kavlock et al., 1982				

Bropirimine – oral - rats

Bropirimine (oral gavage) at 200 or 400 mg/kg bw as single doses on one day during GD 5-18 in rats. (Marks et al., 1990)

Bropirimine (oral gavage) at 400 mg/kg bw during GD 4 with sacrifices on a number of different days (Marks et al., 1990)

Bropirimine (oral gavage) at 0, 25, 50, 100, 200, 400 mg/kg bw/d during GD 7-15 in rats over two experiments (Marks et al., 1988)

Total treatment days	9	1	1	1	1	1	
Day(s) of treatment	7-15	5, 6, 7, 8, 9, 10	11	12, 13, 14, 15, 16, 17	18	4	
Corpora lutea						??/400	
Implantations per dam	400/??						
Pre-implantation loss	400/??					??/400	
Embryonal/foetal resorptions	100/200			400/??	400/??	??/400	
Post-implantation loss				400/??	400/??	??/400	
Foetusses/litter size		400/??	400/??	400/??		??/400	
No. of live fetuses	400/??				??/200	??/400	
Dead foetusses			??/200	400/ ??	??/200		
Sex ratio	400/??						
Body weight (litter)	100/200						
Abnormal foetuses	25/50						
Malformations - External - Visceral Hydronephrosis - Skeletal Delayed ossification Extra rib cervical Misshapen vertebrae	 100/?? 50/100 25/50 25/50 25/50						
Maternal toxicity	25/50	??/200	??/200	??/200	??/200		
	Marks et al., 1988	Marks et al., 1990	Marks et al., 1990	Marks et al., 1990	Marks et al., 1990	Marks et al., 1990	

Butyl Benzyl Phtalate (oral –rat)

Total treatment days	56	21	12	10	9	8	5	3	1
Day(s) of treatment	(-14 premating)-(4 to 6) post partum	0-20 gestation	0-11	0-9 or 11-20 gestation	0-8 or 7-15 gestation	0-7 or 7-16 gestation	16-20 gestation	7-9 or 10-12 or 13-15 gestation	6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 gestation
Corpora lutea	1000/??	974/??	974/??	974/??	1000/??	974/??	974/??		1500/??
Implantations per dam	1000/??	974/??	974/??	974/??	500/750	974/??	974/??	??/750	1500/??
Pre-implantation losses	1000/??	974??	974/??	974/??	750/1000	974/??	974/??		1500/??
Embryonal/foetal resorptions		654/974	??/974	974/??	500/750			600/750	1000/1500
Post-implantation losses	500/1000	654/974	??/974	974/??	500/750	??/974	974/??	600/750	1000/1500
Foetusses/litter size	500/1000	185/375	??/974	974/??	500/750	974/??		600/750	1000/1500
Dead foetusses	500/1000	654/974	??/974	974/??	500/750	??/974	974/??	600/750	
Sex ratio	1000/??	974/??	974/??	974/??	750/1000	974/??	974/??	??/750	1000/1500
Body weight (litter)	250/500	185/375		??/974	250/500	974/??	??/974	600/750	1000/1500
Abnormal foetuses	1000/??	974/??	974/??	??/974	500/750	??/974	974/??	600/750	1000/1500
Malformations - External (a.o. cleft palate) - Visceral (a.o. dilatation of renal pelvis) - Skeletal (a.o. fusion of sternebrae)				 ??/974 974/?? ??/974	 500/750 500/750 500/750	 ??/974 974/?? ??/974		 600/750 1000/?? 600/750	 1000/1500 ??/1500 ??/1000
Maternal Toxicity									
References	Piersma et al., 1995	Ema et al., 1990a; Ema et al., 1990b; Ema et al., 1991b; Ema et al.,	Ema et al. 1992b; Ema et al.,	Ema et al., 1992b; Ema et al., 1994a	Ema et al., 1991a; Ema et al., 1992c; Ema et al., 1998	Ema et al., 1991a; Ema et al., 1992a; Ema et al., 1994a	Ema et al., 1991a; Ema et al., 1992a	Ema et al., 1991a; Ema et al., 1993a; Ema et al.,	Ema et al., 1999

Total treatment days	56	21	12	10	9	8	5	3	1
Day(s) of treatment	(-14 premating)-(4 to 6) post partum	0-20 gestation	0-11	0-9 or 11-20 gestation	0-8 or 7-15 gestation	0-7 or 7-16 gestation	16-20 gestation	7-9 or 10-12 or 13-15 gestation	6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 gestation
		1992a; Ema et al., 1992b; Ema et al., 1994a	1994a					1995	

Cadmium ($CdCl_2$) – various routes - mice

Cadmium (CdCl2, oral drinking water) at 0, 10, 20, 40 mg/l during GD 0-19 in mice (equivalent to 1.4, 2.8, 5.6 mg Cd/kg bw/day) (Webster, 1978)
Cadmium (CdCl2, sc injection) at GD 7 in mice (4, 6 mg/kg bw) (Padmanabhan et al.,, 1986)
Cadmium (CdCl2, sc injection) at GD 7,8, 9, 10, 11, or 12 in mice (2,3,4,6,8,10, 15 mg/kg bw) (Padmanabhan, 1990)
Cadmium (CdCl2, ip injection) at GD 8 (2.0 mg/kg bw) (Sato et al., 1985)
Cadmium (CdCl2, ip injection) at GD 6.5, 7, 7.5, 8, 8.5, 9 at a dose of 4 mg/kg bw (Hovland et al., 1999)

	20	1	1	1	1	
	0-19	7		6.5, 7, 7.5, 8, 8.5, 9	8	
Corpora Lutea						
Placental weight			2/3			
# implantations / dam		6/??	10 (15)/??	4/??	2.0/??	
Pre-implantation loss			10 (15)/??			
Embryonal / foetal resorptions		??/4	??/2 (…..)	??/4		
Post-implantation loss		??/4		??/4		
Foetal / Litter size						
Number of live fetuses			4/6	??/4	2.0/??	
Foetal death			??/2 (…..)	??/4		
Sex ratio						
Body weights (foet/ litter)	??/2.8	4/6	3 / 4 ‡	4/??	??/2.0	
Malformations / Variants					??/2.0	
- external Exencephaly				 ??/4	 ??/2.0	
- visceral						
- skeletal Fused ribs Split vertebrae arches retarded ossification			 ??/4 (…..)	??/4	 2.0/?? ??/2.0	
Maternal toxicity	2.8/5.6		8/10		??/2.0	
	Webster, 1978	Padmanabhan et al., 1986	Padmanabhan, 1990	Hovland et al., 1999	Sato et al., 1985	

‡ Op basis van expert-judgement. Er is nauwelijks een dosis-response relatie voor deze parameter. Inschatting gemaakt door een daling van 0.2 g t.o.v. controle gewicht van 1.6 als relevant te beschouwen.

Cadmium chloride – oral - rats

Cadmium chloride (oral gavage) at 0, 3 [1.8], 10 [6.1], 30 [18.4], 100 [61.3] mg/kg bw/day [Cd 2+] during GD 6-15 in rats (Maechemer et al., 1981)
Cadmium chloride (oral gavage) at 2, 12, 40 mg Cd/kg bw/day
Cadmium acetate (drinking water) at about 21 mg Cd2+/kg bw/day during GD 0-20 in rats (Saxena et al., 1986)
Cadmium acetate (drinking water) at 0.71 and 1.21 mg/kg bw/day during GD 0-20 in rats (Ali et al., 1986)
Cadmium chloride (sc injection) at GD 0-20 at a dose of 0.49 m Cdg/kg bw/day in rats (Hazelhoff Roelfzema, 1988 Thesis)
Cadmiuim chloride (ip injection) at 1.8 mg Cd/kg bw during GD 9, 10, or 11 or (sc injection) during GD 9-11 in rats (Barr, 1973)

	10	10	20	20	20	3 (s.c.)	1 (i.p.)
	6-15	7-16	0-20	0-20	0-20	9-11	9, 10, 11
Corpora Lutea							
Placental weight							
# implantations / dam	18,4/??						
Pre-implantation loss							
Embryonal / foetal resorptions	18.4/61.3	12/40				1.8/??	??/1.8
Post-implantation loss							
Foetal / Litter size			21/??	1.21/??	0.49/??		
Number of live fetuses	18.4/61.3	12/40					
Foetal death	18.4/61.3		21/??	1.21/??	0.49/??		
Sex ratio							
Body weights (foet/ litter)	6.1/18.4	2/12	21/??	0.71/1.21	0.49/??	1.8/??	??/1.8
Malformations / Variants	6.1/18.4	12/40		1.21/??			??/1.8
- external Exencephaly							 ??/1.8
- visceral							
- skeletal Fused ribs Split vertebrae arches retarded ossification		 ??/2	??/21 ??/21				
Maternal toxicity	1.8/6.1	??/2	21/??		??/0.49		
	Maechemer et al., 1981		Saxena et al., 1986	Ali et al., 1986	Hazelhoff Roelfzema, 1988	Barr, 1973	Barr, 1973

Carbendazim – oral – rats

Carbendazim (oral gavage) at 0, 20, 40, 80 mg/kg bw/day during GD 5-15 in rats (Janardhan et al., 1984)

Carbendazim (oral gavage) at 0, 15, 30, 60, 150, 300 mg/kg bw/day during GD 10 in rats (Minta et al., 1982)

	11	10	8	8	1	
	5-15	6-15	1-8	1-8	10	
Corpora Lutea		160/??		1000/??		
# implantations / dam		160/??	400/	1000/??		
Pre-implantation loss						
Embryonal / foetal resorptions	20/40	8/35		1000/??	60/150	
Post-implantation loss		8/35				
Foetal / Litter size		8/35			150/??	
Number of live fetuses	20/40	8/35			60/150	
Foetal death	20/40		100/200			
Sex ratio						
Body weights (foet/ litter)	80/??	8/35	??/100		15/30	
Malformations / Variants	80/??	8/35			150/??	
- external						
- visceral hydronephrosis		 8/35				
- skeletal retarded ossification fused vertebrae/arches fused ribs		 ??/8 8/35 8/35				
Maternal toxicity	80/??	8/35		400/1000	30/60	
	Janardhan et al., 1984		Cummings, 1991	Cummings et al., 1990	Minta et al., 1982	

Carbon tetrachloride – oral - rats

Carbon tetrachloride CCl4 (oral gavage) 150 or 600 mg/kg bw on GD 6, 7, 8, 10, or 12 in rats (Narotsky et al., 1997)
Carbon tetrachloride CCl4 (oral gavage) 0, 150 mg/kg bw on GD 8 in rats (Narotsky et al., 1995)
Carbon tetrachloride CCl4 (oral gavage) 25, 50, 75 mg/kg bw on GD 6-15 in rats (Narotsky et al., 1997)

Treatment days during gestation	GD 6-15	GD 6, 7, 8, 10, 12	GD 8
Number of days	11	1	1
Corpora Lutea		150/??	
# implantations / dam		150/??	
Pre-implantation loss			
Embryonal / foetal resorptions	25/50	??/150	??/150
Post-implantation loss			
Foetal / Litter size			
Number of live fetuses		150/??	
Foetal death		??/150	
Sex ratio			
Body weights (foet/ litter)	75/??	150/??	
Malformations / Variants			
- external			
- visceral			
- skeletal			
Maternal toxicity	25/50	??/150 (bw loss, liver effects)	??/150 (changed hormone levels)
	Narotsky, 1997b In EHC 208	Narotsky et al., 1997	Narotsky et al., 1997

All-or-none effects: either Full Litter Resorption or no embryo/foetotoxicity (Narotsky et al., 1997)

Chlorpropham – oral – mice

Chlorpropham (oral, diet) at 225, 450, 900 mg/kg bw/day in reproduction study in mice (Tanaka et al., 1997a)
Chlorpropham (i.p. injection) at 970 mg/kg bw/day at GD 6-14 (Capariccio et al., 1981)
chlorpropham (oral, gavage) at 050, 1500, or 3000 mg/kg bw during GD 8.3 (Tanaka et al., 1997b)

Total treatment days	Several weeks (repro-study)	9	1
Day(s) of treatment		6-14	8.3 gestation
Corpora lutea			
Implantations per dam			3000/??
Pre-implantation losses			
Embryonal/foetal resorptions		??/970	1500/3000
Post-implantation losses		??/970	1500/3000
Foetusses/litter size	900/??		3000/??
Dead foetusses		??/970	3000/??
Sex ratio	900/??		3000/??
Body weight (litter)	900/??	??/970	1500/3000
Abnormal foetuses		970/??	750/1500
Malformations - External (a.o. cleft palate) - Visceral (a.o. exencephaly and/or brachyury) - Skeletal (a.o. fusion sternebrae)			750/1500
Maternal toxicity			3000/??
	Tanaka et al., 1997a	Capariccio et al., 1981	Tanaka et al., 1997b

Chlorpropham – oral – other species

Chlorpropham (oral, gavage) 0, 50, 200, 800 mg/kg bw/day in rats GD 6-15 (becker and Biedermann, 1990 in JMPR 2000)

Chlorpropham (oral, gavage) 0, 125, 250, 500 mg/kg bw/day in rabbits GD 6-18 (James 1983 in JMPR 2000)

Chlorpropham (oral, gavage) 0, 125, 250, 500 mg/kg bw/day in rabbits GD 6-18 (Waalkens-Berendsen, 1998 in JMPR 2000)

	10 RAT	13 Rabbit	13 Rabbit
	6-15	6-18	6-18
Corpora Lutea	800/??		500/??
# implantations / dam	800/??		500/??
Pre-implantation loss	800/??		500/??
Embryonal / foetal resorptions	800/??	250/500	500/??
Post-implantation loss	800/??	125/250	500/??
Foetal / Litter size	800/??		500/??
Foetal death	800/??		500/??
Sex ratio	800/??	500/??	500/??
Body weights (foet/ litter)	200/800	500/??	250/500
Malformations / Variants			
- external	800/??		
- visceral	800/??	500/??	
- Skeletal Retarded ossification	800/?? 200/800	500/?? 500/??	 250/500
Maternal toxicity	200/800	250/500	125/250
	JMPR 2000 vlgs OECD 414	JMPR 2000 vlgs OECD 414	JMPR 2000 vlgs OECD 414

Di-Butyl Phtalate – oral –rats

Total treatment days	12	11	9	3	1
Day(s) of treatment	0-11 gestation	11-21 gestation	0-8 or 7-15 gestation	7-9, 10-12, 12-14, 13-15, 15-17 or 18-20 gestation	6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 gestation
Corpora lutea	895/??	661/??	1000/??		
Implantations per dam	895/??	661/??	1000/1250	1500/??	
Pre-implantation losses	895/??	661/??	1000/1500		
Embryonal/foetal resorptions	??/895	661/??	500/630	750/1000	1000/15000
Post-implantation losses	??/895	661/??	500/630	??/750	??/1500
Foetusses/litter size	??/895	661/??	500/630	??/750	??/1500
Dead foetusses	??/895	661/??	500/630	??/750	
Sex ratio	895/??	661/??	500/630	750/1000	??/1500
Body weight (litter)	895/??	??/661	??/250	??/750	1000/15000
Abnormal foetuses	895/??	??/661	630/750	??/750	500/1000
Malformations - External (a.o. cleft palate) - Visceral (a.o. dilatation of renal pelvis) - Skeletal (a.o. fusion of sternebrae)		??/661 ??/555 ??/661	630/750	??/750 1000/?? ??/750	??/1500 ??/1500 500/1000
Maternal Toxicity	??/895	??/555	250/500	500/1000	1000/1500
References	Ema et al., 1997a	Ema et al., 1998a	Ema et al., 1993a, b; 2000b	Ema et al, 1993a; 1994; 2000a	Ema et al,. 1998b, 1997b, Saillenfait et al,. 1998

Deltamethrin – oral - mice

Deltamethrin (oral gavage) at doses of 3, 6, 12 mg/kg bw/day during GD 7-16 in mice (Kavlock et al., 1976; in JMPR 2000)

Deltamethrin (oral gavage) at doses of 30, 38 mg/kg bw during GD 8 in mice (Kavlock et al., 1985)

Total days of treatment	10	1
GD	7-16	8
Corpora Lutea		
# implantations / dam	12/??	
Pre-implantation loss		
Embryonal / foetal resorptions	12/??	
Post-implantation loss	12/??	38/??
Foetal / Litter size		
Number of live fetuses		
Foetal death		
Sex ratio		
Body weights (foet/ litter)	12/??	38/??
Malformations / Variants	12/??	38/??
- external		
- visceral		
- skeletal delayed ossification supernumary ribs	 12/?? 6/12	 38/?? 38/??
Maternal toxicity	3/6	30/38
	Kavlock et al., 1976 in JMPR 2000	Kavlock et al., 1985

Ethylene thiourea (ETU) – oral - hamster

Ethylenethiourea (oral, gavage) 0, 90, 270, 810 mg/kg bw/day during organogenesis in Syrian Hamster (Teramoto et al. 1978)
Ethylenethiourea (oral, gavage) 0, 600, 1200, 1800, 2400 mg/kg bw/day GD 11 in Syrian Hamster (Khera et al., 1983)

	8	1	1
	6-13	11	
Corpora Lutea			
# implantations / dam	810/??		
Pre-implantation loss	810/??		
Embryonal / foetal resorptions		1800/2400	
Post-implantation loss		1800/2400	
Foetal / Litter size			
Number of liver fetuses		1800/2400	
Foetal death	270/810	1800/2400	
Sex ratio	810/??		
Body weights (foet/ litter)	90/270	600/1200	
Malformations / Variants		600/1200	
- external (e.g. cleft palate)	90/270	600/1200	
- visceral (hydrocephalus, hypoplastic cerbellum)	270/810	600/1200	
- Skeletal (various) Retarded ossification (calvarium)	90/270 (not reported)	600/1200 600/1200	
Maternal toxicity	810/??	2400/??	
	Teramoto et al., 1978	Khera et al., 1983	

Ethylene thiourea (ETU) – oral – rat

Ethylenethiourea (oral intubation) in rats on GD 6-15 (0, 10, 20, 30, 40, 50 mg/kg bw). (Teramoto et al., 1978)
Ethylenethiourea (oral, intragastric) in rats on GD 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 (Hung et al., 1986)

	10	10	1
	6-15	6-15	8 or 9 (other days are less important)
Corpora Lutea			??
# implantations / dam	50/??		??
Pre-implantation loss	50/??		
Embryonal / foetal resorptions			??/60
Post-implantation loss			
Foetal / Litter size			
# live fetuses	50/??		
Foetal death	40/50		
Sex ratio	50/??		
Body weights (foet/ litter)	20/30	40/80	
Malformations / Variants			
- external cephalic malf.	20/30	5/10 10/20	??/60
- visceral	10/20		
- skeletal Retarded ossification	20/30		
Maternal toxicity	50/??	40/80	240/??
	Teramoto, 1978	Khera KS, 1973 In: Piersma et al., (2002) in prep.	Hung et al., 1986

FM-100 (flame retardant)– inhalation - rats

FM-100™ (inhalation) at levels 1000, 4000, 10000 ppm (6h/day) during GD 6-15 in rats (Nemec et al., 1992a)

FM-100™ (inhalation) at levels 20000, 30000, 40000 ppm (6h/day) during GD 9 in rats (Nemec et al., 1992b)

‘	10		1			
	6-15		9			
Corpora Lutea						
# implantations / dam						
Pre-implantation loss						
Embryonal / foetal resorptions						
Post-implantation loss						
Foetal / Litter size						
Number of live fetuses						
Foetal death						
Sex ratio						
Body weights (foet/ litter)	4000/10000		40000/??			
Malformations / Variants	4000/10000		20000/30000			
- external microphthalmia	 4000/10000		 40000/??			
- visceral						
- skeletal reduced ossification 7th cervical rib	 4000/10000 4000/10000		 20000/30000			
Maternal toxicity	4000/10000		??/20000			
	Nemec et al., 1992a		Nemec et al., 1992b			

Manganese – subcutane injection - mice

Manganese chloride tetrachloride (s.c. injection) at 50 mg/kg bw during GD 9, 10, 11, or 12 in mice (Colomina et al.,1996)
Manganese chloride tetrachloride (s.c. injection) at 2, 4, 8, 16 mg/kg bw/day during GD 6-15 in mice (Sanchez et al., 1993)

Total treatment days	10	1	1	
Day(s) of treatment	6-15	9,10 gestation	11, 12 gestation	
Corpora lutea				
Implantations per dam	16/??	50/??	50/??	
Pre-implantation losses				
Embryonal/foetal resorptions	2/4	??/50	50/??	
Post-implantation losses		??/50	??/50	
Foetusses/litter size		??/50	??/50	
Dead foetusses	16/??	50/??	50/??	
Sex ratio	16/??	50/??	50/??	
Body weight (litter)	4/8	??/50	??/50	
Abnormal foetuses		??/50	??/50	
Malformations - External - Visceral renal hypoplasia - Skeletal reduced ossification wavy ribs	 16/?? 16/?? 8/16 2/4 8/16	 50/?? 50/?? ??/50 ??/50	 50/?? 50/?? ??/50	
Maternal toxicity	4/8			
		Colomina et al., 1996	Colomina et al., 1996	

Methanol – oral -rats

Methanol (Oral, drinking water) 350, 700, 1400 mg/kg bw in rats (folate deficient diet) (Ryan, 1994) and Methanol (Oral, drinking water) 357, 714, 1428 mg/kg bw in rats (folate deficient diet) (IUCLID ref.328)
Methanol (Oral, gavage) 2500 mg/kg bw in rats (well noursihed) (De Carvalho, 1994); Methanol (Oral, gavage) 2500 mg/kg bw in rats (??)
Methanol (Oral, gavage) 4000 mg/kg bw in rats (Rogers et al., 1993)
Methanol (oral gavage) 1040, 2080, 4240 mg/kg bw (Youssef, 1991; Youssef et al., 1997)

Number of treatment days	10	10	10	10	5	1
Days of gestation	6-15	6-15	6-15	6-15	6-10	10
Corpora Lutea			2500/??			4240/??
# implantations / dam			2500/??	??/4000		4240/??
Pre-implantation loss			2500/??			
Embryonal / foetal resorptions	350/700	357/714	2500/??	??/4000		
Post-implantation loss			2500/??			4240/??
Foetal / Litter size						4240/??
Foetal death	350/700		2500/??	??/4000		
Sex ratio						4240/??
Body weights (foet/ litter)			??/2500		??/2500	??/1040
Malformations / Variants				??/4000		1040/2080
- External				??/4000		
- Visceral Neural tube defects						2080/4280
- Skeletal Delayed ossification Supernumary ribs	350/700		??/2500 ??/2500 ??/2500		??/2500	4280/??
Maternal toxicity	350/700	357/714	2500/??	??/4000		2080/4240
	Ryan, 1994	Interim report in IUCLID (328) ??	De Carvalho, 1994	Rogers et al., 1993		Yousef, 1991; Yousef et al., 1997

Methanol – inhalation - mice

Methanol (inhalatoir) 0, 5000, 10000, 15000 ppm 6h/day during GD 6-15, GD 7-9, GD 9-11, or GD, 7-8, 8-9, or GD 7, 8, 9 (Bolon et al., 1993)

Number of treatment days	10	3	3	2	2	1	1	1
Days of gestation	6-15	7-9	9-11	7-8	8-9	7	8	9
Corpora Lutea								
# implantations / dam	15000/??	15000/??	15000/??	15000/??	15000/??	15000/??	15000/??	15000/??
Pre-implantation loss								
Embryonal / foetal resorptions	5000/10000	5000/10000	15000/??	10000/15000	15000/??	10000/15000	15000/??	15000/??
Post-implantation loss								
Foetal / Litter size								
Foetal death	10000/??	10000/15000	15000/??	??/15000	15000/??	??/15000	15000/??	15000/??
Sex ratio								
Body weights (foet/ litter)	??/10000	10000/15000	15000/??	10000/15000	15000/??	15000/??	15000/??	15000/??
Malformations / Variants								
- External Neural tube defects Cleft palate Ocular effects	 ??/10000 ??/10000	 5000/10000 5000/10000 5000/10000	 15000/?? ??/10000 15000/??	??/15000	??/15000	15000/??	??/15000	15000/??
- Visceral Renal pelvic dilation a)		 ??/5000	 5000/??					
- Skeletal Digit malformations	 ??/10000	 10000/??	 ??/10000					
Maternal toxicity	5000/10000	10000/15000		10000/15000		10000/15000		
	Bolon et al., 1993							

a) also present in significant quantities in controls.

Methanol (inhalation) 0, 1000, 2000, 5000, 7500, 10000, 15000 ppm 7h/day, from GD 6-15 in mice (Rogers et al., 1993)
Methanol (inhalation) 10000 ppm 6h/day, GD 8 in mice (Dorman et al., 1995 in IUCLID ref. No. 325)

Number of treatment days	12	1		
Days of gestation	6-15	8		
Corpora Lutea	15000/ ??			
# implantations / dam	15000/??			
Pre-implantation loss				
Embryonal / foetal resorptions	5000/7500			
Post-implantation loss				
Foetal / Litter size				
Foetal death	5000/7500			
Sex ratio				
Body weights (foet/ litter)	7500/10000	10000/??		
Malformations / Variants	1000/2000			
- External Exencephaly Cleft palate Neural tube defects	 2000/5000	 ??/10000		
- Visceral	2000/5000			
- Skeletal Cervical ribs Delayed ossification	 1000/2000 2000/5000			
Maternal toxicity	5000/7500			
	Rogers et al., 1993	Dorman et al., 1995 (IUCLID 325)		

Summary of mice inhalation studies (in ppm)

Total treatment days	10	3	3	2	2	1	1
Day(s) of treatment	6-15	7-9	9-11	6-7 or 7-8	8-9, 9-10, 10-11, 11-12, 12-13	5, 6, 7	8, 9
Corpora lutea	15000/??						
Implantations per dam	15000/??	15000/??	15000/??	15000/??	15000/??	15000/??	15000/??
Pre-implantation losses	15000/??						
Embryonal/foetal resorptions	5000/7500	5000/10000	15000/??	10000/15000	15000/??	??/10000	15000/??
Post-implantation losses							
Foetusses/litter size							
Dead foetusses	5000/7500	10000/15000	15000/??	5000/10000 ?	15000/??	5000/10000	15000/??
Sex ratio							
Body weight (litter)	7500/10000	10000/15000	15000/??	5000/10000	15000/??	5000/10000	15000/??
Abnormal foetuses	1000/2000						
Malformations							
- External							
Exencephaly	2000/5000					??/10000	??/10000
Cleft palate	2000/5000	5000/10000	??/10000	??/10000		5000/10000	5000/10000
Neural tube defects	7500/10000	5000/10000	15000/??	??/10000	??/15000	15000/??	??10000
- Visceral							
- Skeletal		10000/??	10000/??				
Cervical / lumbar ribs	1000/2000			??/10000	??/10000	2000/5000	??/10000
Retarded ossification	1000/2000						
Maternal toxicity		10000/15000	10000/15000	10000/15000	10000/??	10000/15000	
References	Bolon et al. 1993; Rogers et al. 1991, 1993	Bolon et al. 1993		Bolon et al. 1993; Rogers and Mole 1997			

Methanol – inhalation – rats

Methanol (inhalation) 0, 5000, 10000, 20000 ppm 7h/day, GD 7-15 (low and mid-dose 1-19) or 0, 200, 1000, 5000 ppm (24h/day) GD 7-17 in rats (IUCLID database on Methanol).

Number of treatment days	11	9	8
Days of gestation	7-17	7-15	3-14
Corpora Lutea		20000/??	
# implantations / dam		20000/??	
Pre-implantation loss		20000/??	
Embryonal / foetal resorptions		20000/??	?
Post-implantation loss		20000/??	
Foetal / Litter size			
Foetal death	1000/5000	20000/??	1000/5000
Sex ratio	5000/??		
Body weights (foet/ litter)	1000/5000	5000/10000	1000/5000
Malformations / Variants		10000/20000	
- External			
- visceral heart		10000/20000 (of lager ?)	1000/5000
- skeletal retarded ossification cervical ribs		10000/20000	1000/5000 1000/5000
Maternal toxicity	1000/5000	10000/20000	1000/5000
	Refs 317 IUCLID NEDO 1987	(refs 315/316 IUCLID) Nelson et al., 1985	NEDO study, cited in Marcus, 1993.

2-Methoxy ethanol – oral - mice

2-Methoxyethanol (gastric intubation) 31.25, 62.5, 125, 250, 500, 1000 mg/kg bw) day 7-14 gestation in mice (Nagano et al., 1981)

2-Methoxyethanol (oral, gavage) 250 mg/kg bw/d during GD 7-9, 8-10, 9-11, 7-8, 9-10, or 10-11; 500 mg/kg bw on GD 9, 10, 11, 12, or 13; or 100, 175, 250, 300, 350, 400, or 450 mg/kg bw on GD 11 in mice (Horton et al., 1985)

Number of treatment days	7 days	3	3	2	1	1
Days of gestation	Day 7-14	7-9	8-10, 9-11	7-8, 9-10, 10-11	9, 10	11, 12, 13
Corpora Lutea						
# implantations / dam	1000/??					
Pre-implantation loss						
Embryonal / foetal resorptions	62.5/125	??/250	??/250	??/250	??/500	450/500
Post-implantation loss	62.5/125	??/250	??/250	??/250	??/500	450/500
Foetal / Litter size						
Number of viable fetuses	125/250	??/250	250/??	250/??	??/500	500/??
Foetal death	125/250	??/250	250/??	250/??	??/500	500/??
Sex ratio						
Body weights (foet/ litter)	62.5/125	??/250	??/250	??/250	??/500	175/250
Malformations / Variants	??/31.25					
- external exencephaly	62.5/125	??/250	??/250	??/250		
- visceral						
- skeletal umbilical hernia abnormal digits delayed ossification Fused ribs Spina bifida	31.25/62.5 125/250 125/250 ??/31.25 62.5/125 31.25/62.5	??/250 ??/250	??/250 ??/250	??/250 ??/250	??/500 ??/500	100/175 ??/500
Maternal toxicity	125/250 (bw)	250/??	250/??	250/??	500/??	500/??
	Nagano et al., 1981	Horton et al., 1985	Horton et al., 1985	Horton et al., 1985	Horton et al., 1985	Horton et al., 1985

2-Methoxyethanol – oral -rats

2-methoxyethanol (oral, gavage) 12.5, 25, 50, 100 mg/kg bw/d in rats (IUCLID 229/230); 2-methoxyethanol (oral, gavage) 50, 100 mg/kg bw/d in rats (IUCLID 231); 2-methoxyethanol (oral gavage) 25, 50 or 100 mg/kg bw/d in rats (IUCLID 232: Toraason et al., 1985); 2-methoxyethanol (oral gavage) 25, 50, 75 mg/kg bw/d in rats (IUCLID 233)

2-methoxyethanol (oral, gavage) 0, 25, 50, 75, 100, 125, 150 mg/kg bw/d in rats (Nelson et al., 1994)

2-methoxyethanol (oral, gavage) 0, or 75 mg/kg bw/d in rats (Narotsky et al., 1993)

2-methoxyethanol (oral, gavage) 0, 250 mg/kg bw/d in rats on GD 13 (Sleet et al., 1994)

	10	7	7	7	7	4, 7, 10	2	1	1	1
	6-15	9-15	7-13	7-13	6-12	6-9, 6-12, 6-15	6-7	9	13	13
Corpora Lutea						75/??				
# implantations / dam			25/50							
Pre-implantation loss										
Embryonal / foetal resorptions	25/50		25/50	??		??/75	75/??	100/125		150/??
Post-implantation loss			25/50							
Foetal / Litter size				??/50	25/50					
Foetal death	12.5/25	??/50	50/??	??/50	25/50					
Sex ratio										
Body weights (foet/ litter)	50/100	??/50 ??	25/50	??/50	25/50	75/??	75/??	150/??		125/150
Malformations / Variants		??/50		??/50				125/150		125/150
- external		Nd								125/150
- Visceral Cardiovascular effects Hydronephrosis		??/50	??/25 25/50 ??/25	??/50						
- skeletal Malformed paws		Nd	nd						??/250 ??/250	
Maternal toxicity	50/100	50/100	25/50	50/75 ??	75/??					
	IUCLID 229, 230	IUCLID 231	IUCLID 232 Toraason et al., 1985	IUCLID 233	IUCLID 234	Narotsky et al., 1993	Narotsky et al., 1993	IUCLID 241/242 Nelson et al., 1994	Sleet et al.,	IUCLID 241/242 Nelson et al., 1994

IUCLID 235: 25 mg/kg bw/d GD 7-13 of 13-19 is NOAEL (litter size, litter weight, foetal weight, enzyme activity in heart of foetuses)

2-Methoxyethanol (oral, gavage) 0, 25, 50, 75, 100, 125, 150 mg/kg bw/d in rats (Nelson et al., 1994)

	1	1					
	9	13					
Corpora Lutea							
# implantations / dam							
Pre-implantation loss							
Embryonal / foetal resorptions	100/125	150/??					
Post-implantation loss							
Foetal / Litter size							
Foetal death							
Sex ratio							
Body weights (foet/ litter)	150/??	125/150					
Malformations / Variants	125/150	125/150					
- external		125/150					
- Visceral Cardiovascular effects Hydronephrosis							
- skeletal							
Maternal toxicity							
	IUCLID 241/242 Nelson et al., 1994	IUCLID 241/242 Nelson et al., 1994					

2-Methoxyethanol – dermal – rats

2-Methoxyethanol (Dermal, 6h/day) 483, 773, 966 mg/kg bw/d GD 6-15 in rats (IUCLID ref. 257)

2-methoxyethanol (Dermal, 6h/day) 48, 97, 290 mg/kg bw/day GD 6-15in rats (IUCLID ref. No. 258)

2-methoxyethnaol (Dermal, test substance was not removed) at 0, 250, 500, 1000 mg/kg bw (Feuston et al., 1990)

2-methoxyethanol (s.c. injection) 150, 400 mg/kg bw/d GD 7-14 in mice

	10 dermal rat	10 dermal rat	1	1	8 (s.c.) mice
	6-15	6-15	12	10, 11, 13, 14	7-14
Corpora Lutea			2000/??		
# implantations / dam			2000/??		
Pre-implantation loss					
Embryonal / foetal resorptions	??/483	48/97	2000/??	??/2000	
Post-implantation loss	??/483	48/97			
Foetal / Litter size			2000/??	2000/??	
Foetal death	??/483	48/97	2000/??	2000/??	150/400
No. of live fetuses			500/1000		
Sex ratio					
Body weights (foet/ litter)		??/48	500/1000	??/2000	??/150
Malformations / Variants		??/48	250/500	??/2000	??/150
- external cleft palate		??/48	250/500 500/1000		
- Visceral Cardiovascular effects		??/48	250/500		
- skeletal retarded ossification		??/48 ??/48	250/500 250/500		
Maternal toxicity	??/483	48/97	500/1000	??/2000	??
	IUCLID 257 OECD 414	IUCLID 258 OECD 414			

Nickel ($NiCl_2$) – parenteral injection (i.m./ i.p) – rats

Nickel chloride (i.m. injection) 1.5, 2.0 mg Ni/kg bw twice a day (=3 and 4 mg/kg bw/day) at day 6-10 or 8, 12, 16 mg Ni/kg bw at day 8, or 6, 8, 16 mg Ni/kg bw on day 18 in rats.

Nickel chloride (i.p. injection) at 1, 2, 4 mg Ni/kg bw during GD 8, 12, or 16 in rats (Mas et al., 1985)

Number of treatment days	5	1	1	1
Days of gestation	6-10	8	18	8, 12, 16
Corpora Lutea	4/??	16/??		
# implantations / dam			16/??	
Pre-implantation loss				
Embryonal / foetal resorptions				4/??
Post-implantation loss				4/??
Foetal / Litter size				
Foetal death	3 / 4	8/12	8/16	4/??
Sex ratio				
Body weights (foet/ litter)	4/??	12/16		1 / 2
Malformations / Variants	4/??	16/??		
- External hydrocephalus				??/1
- visceral hydronephrosis				??/1
- skeletal retarded ossification				1/2
Maternal toxicity		LD5 is about 17 mg/kg bw	8/16 (death)	
	Sunderman et al., 1978	Sunderman et al., 1978	Sunderman et al., 1978	Mas et al., 1985

Nickel ($Ni(CO)_4$) – inhalation – rat & hamster

Nickel (as nickelcarbonyl) by inhalation: 8.6 ppm Ni(CO)4 for 15 min on GD 5 in hamsters (Sunderman et al., 1980)

Nickel (as nickelcarbonyl) by inhalation 11.2, 22, 42 ppm for 15 min on GD 7 in rats (Sunderman et al., 1979)

Number of treatment days		1	1	1	1
Days of gestation		7, 8, 9	5	4 or 5	6, 7 or 8
Corpora Lutea		42/??		8.6/??	8.6/??
# implantations / dam				8.6/??	8.6/??
Pre-implantation loss					
Embryonal / foetal resorptions				??/8.6	8.6/??
Post-implantation loss					
Foetal / Litter size					
Foetal death		22/42	??/8.6	??/8.6	8.6/??
Sex ratio					
Body weights (foet/ litter)		11/22	??/8.6 (bw increased)	8.6/??	8.6/??
Malformations / Variants		??/11	No malformations ?	??/8.6	??/8.6
- External Exencephaly Cleft palate Eye defects		 ??/11		 ??/8.6 ??/8.6	 8.6/?? 8.6/??
- visceral					
- skeletal Fused ribs				 ??/8.6	 8.6/??
Maternal toxicity			??/8.6 ppm (death)	??/8.6	??/8.6
		Sunderman, 1979 (rats)		Sunderman, 1980 (hamster)	Sunderman, 1980 (hamster)

Paclobutrazole – oral – rats

Paclobutrazol (oral gavage) at 50, 200 or 500 mg/kg bw/day during GD 6-15 or on GD 7, 9, 11, or 13 in rats (Vergieva, 1998)

Total treatment days	10	1	1	1	1
Day(s) of treatment	6-15 gestation	7 gestation	9 gestation	11 gestation	13 gestation
Corpora lutea	200/??	500/??	500/??	500/??	500/??
Implantations per dam					
Pre-implantation losses					
Embryonal/foetal resorptions	200/??	500/??	500/??	500/??	500/??
Post-implantation losses					
Foetusses/litter size	200/??	500/??	500/??	500/??	500/??
Dead foetusses					
Sex ratio					
Body weight	200/??	500/??	500/??	500/??	500/??
Abnormal foetuses	50/200	500/??	200/500	??/200	200/500
Malformations - External (a.o. cleft palate) - Visceral (a.o. dilatation of renal pelvis) - Skeletal (a.o. fusion sternebrae)	50/200		200/500	??/200	200/500
Maternal toxicity	??/50				
	Vergieva, 1998	Vergieva, 1998	Vergieva, 1998	Vergieva, 1998	Vergieva, 1998

Phenytoin – oral – rats

Phenytoin (oral gavage) at 0, 150, 375, 750, 1125, 1500 mg/kg bw/day during GD 8 –17 in rats (Rowland et al., 1990)
Phenytoin (oral gavage) at 150 mg/kg bw at GD 11 in rats (Danielsson et al., 2000)
Phenytoin (oral gavage) at 0, 500, 1500 mg/kg bw at GD 10 in rats (Beekhuijzen et al., 2000)
Phenytoin (oral gavage) at 1000 mg/kg bw during GD 9, 11, or 13 (cited in Danielsson et al., 2000)
Phenytoin (oral gavage) at 0, 200, 250 mg/kg bw/day during GD 7-18 (cited in Danielsson et al., 2000)

Number of treatment days	10	12	1	1	1	
Days of treatment (GD)	8-17	7-18	10	11	9, 11, 13	
Corpora Lutea			1500/??			
# implantations / dam			1500/??			
Pre-implantation loss			1500/??			
Embryonal / foetal resorptions	750/1125		500/1500	150/??		
Post-implantation loss						
Foetal / Litter size	375/750					
Number of live fetuses			1500/??			
Foetal death	150/375 750/1125	200/250	1500/??	150/??		
Sex ratio						
Body weights (foet/ litter)	150/375	??/200	500/1500	150/??	??/1000	
Malformations / Variants	375/750		??/500			
- External craniofacial	375/750	250/??	??/500	150/??	1000/??	
- visceral cadiovascular hydronephrosis	375/750 375/750		500/1500			
- skeletal reduced ossification fused vertebrae / ribs	750/1125 375/750		500/1500 500/1500		??/1000	
Maternal toxicity	150/375		500/1500	150/??		
	Rowland et al., 1990	Voorhees et al., 1983	Beekhuijzen et al., 2000	Danielsson et al., 2000	Lorente et al., 1981	

Retinylpalmitate – oral rats

Retinylpalmitate (oral, gavage, rapeseed oil) at 30, 90 mg/kg bw/day during GD 6-15 in rats (Kistler, 1979 in: Collins et al., 1994)
Retinyl palmitate (oral gavage (olive oil)) at 100, 300 or 1000 mg/kg bw/day during GD 10 in rats. (Piersma et al., 1996ab, Olling et al., 1995)
Retinyl palmitate (oral gavage) at 1000 mg/kg bw/day during GD 9-10 in rats (Levine et al., 1999)

Total treatment days	11	2	1	1
Day(s) of treatment	6-15	9-10	10 or 11	9
Corpora lutea				
Implantations per dam	90/??	1000/??	1000/??	
Pre-implantation losses		1000/??		
Embryonal/foetal resorptions	90/??	??/1000	100/300	330/??
Post-implantation losses	90/??	??/1000	100/300	
Foetusses/litter size				330/?? IU
Dead foetusses	90/??		300/1000	
Sex ratio				
Body weight (litter)	90/??	??/1000		330/?? IU
Abnormal foetuses	??/30	??/1000	100/300	
Malformations				
- External			100/300	
Cleft palate	30/90	??/1000	100/300	165/330 IU
Exencephaly	30/90	??/1000	100/300	
Abnormal ear				??/165
- Visceral				
(a.o. dilatation of renal pelvis)		??/1000	100/300	
- Skeletal	??/30	??/1000		
no. of somites				
Maternal toxicity				
	Collins et al., 1994	Levine et al., 1999	Piersma et al., 1996a; Piersma et al., 1996b; Olling et al.., 1995	

Thiabendazole – oral - mice

Thiabendaziole (oral gavage) at 700, 1300, or 2400 mg/kg bw/day during GD 7-15 or at 10, 25, 30, 40, 60, 62, 80, 100, 120, 129, 200, 240, 269, 480, 558, 670, 700, 804, 965, 1157, 1300, 1389, 1667, 2000 or 2400 mg/kg bw/day at GD 9 (other GDs only 2400 mg/kg bw was tested) (Ogata et al., 1984).

Total treatment days	9	10	1	1
Day(s) of treatment	7-15 gestation	6-15	6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 gestation	9
Corpora lutea	2400/??		2400/??	
Implantations per dam	2400/??	25/100	2400/??	1000/??
Pre-implantation losses				1000/??
Embryonal/foetal resorptions	700/1300		1389/1667	1000/??
Post-implantation losses				1000/??
Foetusses/litter size			1667/2000	
No. of viable fetuses				1000/??
Dead foetusses	700/1300		2400/??	1000/??
Sex ratio				1000/??
Body weight (litter)	??/700	25/100	30/60	500/1000
Abnormal foetuses	??/700	25/100	129/240	??/250
Malformations				
- External	??/700		965/1157	250/500
limb deformation				250/500
cleft palate	??/700		??/2400	
- Visceral				
	700/1300		129/240	??/250
- Skeletal		25/100		??/250
digit malformations				
fused ribs	2400/??		129/240	
Maternal Toxicity	??/700	25/100	965/1157	
References	Ogata et al., 1984	Nakatsuka et al., 1995	Ogata et al., 1984	

Thiabendazole – oral – rats

Thiabendazole (oral gavage) at 10, 40, 80 mg/kg bw/day during GD 6-17 in rats (Wise, 1990)

Thiabendazole (oral gavage) at 25, 100, 200 mg/kg bw/day during GD 6-15 in rats (Nakatsuka et al., 1995)

Total treatment days	12	10		
Day(s) of treatment	6-17 gestation	6-15 gestation		
Corpora lutea				
Implantations per dam		25/100		
Pre-implantation losses				
Embryonal/foetal resorptions	80/??			
Post-implantation losses				
Foetusses/litter size				
Dead foetusses				
Sex ratio				
Body weight (litter)	10/40	25/100		
Abnormal foetuses	80/??	25/100		
Malformations - External (a.o. cleft palate) - Visceral (a.o. dilatation of renal pelvis) - Skeletal (a.o. fusion of sternebrae, delayed ossification)		25/100		
Maternal Toxicity	10/40	25/100		
References	Wise, 1990	Nakatsuka et al., 1995		

Tributyltin chloride – oral - gavage

Tributyltin chloride (100 or 200 mg/kg bw/day) in rats (Ema et al., 1997)
Tributyltin chloride (oral, gavage) 3.1, 6.3, 9.4, 12.5 mg/kg bw/d on GD 7-9 or 6.3, 9.4, 12.5 mg/kg bw/d on GD 10-12 in rats
Tributyltinoxide (oral, gavage) 1, 4, 6, 12, 23, 35 mg/kg bw/d on GD 6-15 in rats (ORTEP publication)
Tributyltinoxide (oral, gavage) 5, 9, 18 mg/kg bw/d on GD 6-19 in rats (Sheldon in ORTEP publication)
Tributyltin chloride (oral gavage) at 0, 5, 9, 15 mg/kg bw/day during GD 7-15 in rats (Itami et al., 1990)

Total treatment days	14	11	9	3	3	1	1	1	1	1	1
Day(s) of treatment	9-19	6-15	7-15	GD 7-9	GD 10-12	7 gestation	8 gestation	9 gestation	10 gestation	GD 11, 12, 13, 14	15 gestation
Corpora lutea	18/??										
Implantations per dam	18/??			12.5/??	12.5/??	200/ ??	200/ ??	200/ ??	200/ ??	200/ ??	200/ ??
Pre-implantation losses				12.5/??							
Embryonal/foetal resorptions	9/18	23/35	15/??	3.1/6.3	6.3/9.4	100/ 200	100/ 200	100/ 200	200/ ??	200/ ??	200/ ??
Post-implantation losses	9/18			3.1/6.3	6.3/9.4	??/ 100	??/ 100	??/ 100	??/ 200	??/ 200	200/??
Foetusses/litter size						100/ 200	100/ 200	??/ 100	200/ ??	200/??	200/??
Dead foetusses		23/35	15/??	3.1/6.3	6.3/9.4						
Sex ratio	18/??		15/??	21.5/??	21.5/??	100/ 200	200/ ??	100/ 200	200/ ??	200/??	200/??
Body weight (litter)	9/18	12/23	??/5	12.5/??	9.4/12.5	??/ 100	200/ ??	??/ 100	??/ 200	??/ 200	??/ 200
Abnormal foetuses	5/9	1 /4	15/??		??/6.3	200/ ??	??/ 100	200/ ??	200/ ??	??/ 200	200/ ??
Malformations - External cleft palate - Visceral hydronephrosis - Skeletal delayed ossification	 9/18 9/18 9/18	 1 /4		 6.3/9.4 6.3/9.4 12.5/??	 ??/6.3 12.5/?? ??/6.3 12.5/??		 100/ 200			 ??/ 200	
Maternal toxicity	9/18	6/12			3.1/	??/ 100	??/ 100	??/ 100	??/ 200	??/ 200	??/ 200
	TBTO	TBTO ORTEP publication	Itami et al., 1990	Ema et al., 1999	Ema et al., 1999	Ema et al., 1997	Ema et al., 1997	Ema et al., 1997	Ema et al., 1997	Ema et al., 1997	Ema et al., 1997

Trichloroethylene – oral - rats

Trichloroethylene (oral gavage) at 0, 475, 633, 844, 1125 mg/kg bw/day on GD 6-15 (Narotsky et al., 1995)
Trichloroethylene (oral gavage) at 0, 960, or 1280 mg/kg bw/d on GD 8-9 in rats (Narotsky et al., 1999)

	10	2
	6-15	8-9
Corpora Lutea		
# implantations / dam		
Pre-implantation loss		
Embryonal / foetal resorptions	320/475	960/1280
Post-implantation loss	320/475	
Foetal / Litter size		
Number of live fetuses		
Foetal death		??/960
Sex ratio		
Body weights (foet/ litter)	844/1125	
Malformations / Variants		
- external eye defects	475/633	
- visceral		
- skeletal		
Maternal toxicity	320/475	
	Narotsky et al., 1995	Narotsky et al., 1999