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MINI REVIEW

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Fundamentals and possibilities of classification of occupational substances as developmental toxicants

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Abstract It is now widely accepted that describing and labeling of chemicals as developmental toxicants on a purely qualitative basis does not make sense. Agents possessing the potential to induce reproductive or developmental toxicity present a risk of human harm only under certain conditions. This critical fact cannot be properly communicated with a simple designation as "positive" or "negative". Rather, a number of parameters that deal with dose or concentration, frequency, duration and route of exposure must also be conveyed. Unsubstantiated blacklisting is equally counterproductive for preventive medicine as downplaying of the toxicity of chemicals. Gender-based restrictions on exposure at workplaces of women of childbearing age are neither socially acceptable nor scientifically justifiable. Therefore, the German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area published in 1983 a quantitatively based classification concept, which became effective in 1985 and was modified in the following years. The present contribution summarizes what is required for an integrated judgment on the relevance of laboratory and epidemiological data for predicting the potential risk associated with exposure at workplaces to occupational chemicals. Methyl mercury, carbon disulfide, dimethylformamide, ethanol, toluene, *N*,*N*-dimethyl acetamide, nitrous oxide, methanol, ethyl benzene, and phosphorus pentoxide will be described as examples of classified substances.

Key words Developmental toxicants · Classification scheme

Introduction

Several thousand developmental toxicants have been identified in laboratory animals, whereas only about 50 have demonstrated this proclivity in the human species (Schardein and Keller 1989). This tremendous difference is certainly not sufficiently explained by differences in species susceptibility. Rather it is due to either absence of exposure or exposure to lower concentrations. Difficulties in demonstrating associations or proving causation in the human because of confounders and low power are additional reasons for these differences (Schardein et al. 1985). Developmental toxicity data reported in the literature are often unevaluated, incomplete, and inconclusive. This is perhaps one of several reasons why regulatory agencies worldwide have so far not proposed a quantification scheme. For example, a current Directive of the European Union that became effective in 1993 provides a classification of reproductive toxicants into three categories (EEC 1993 a):

1. Substances known to cause developmental toxicity in humans

2. Substances which should be regarded as if they cause developmental toxicity to humans

3. Substances which cause concern for humans owing to possible developmental toxic effects.

Substances which do not meet the criteria specified in detail for the three categories must not be classified, as, for example, is the case when adequate evidence exists to show that the metabolite or mode of action responsible for induction of developmental toxicity is not produced in or is not relevant to man.

Although this regulation is science-based because it requests state of the art investigations as a prerequisite for classification and because it calls for consideration of the dose-response effects, it remains qualitative rather than quantitative. Similarly, the guidelines for developmental toxicity risk assessment of the U.S. EPA (1991) are not yet quantitatively based but the agency has committed itself to development of an additional approach for more quantitative dose-response evaluation (Vandenberg 1994). Therefore, the need for a quantitatively based classification concept is evident.

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Definition and detection of developmental toxicants

Karrh et al. (1981) defined an embryo-fetotoxin, now generally called a developmental toxicant, as a chemical which manifests an effect upon the conceptus during any of the stages of gestation, from fertilization to birth. It may induce death, structural malformations, metabolic or physiological dysfunction, growth retardation, or psychological and behavioral alterations which may be manifest at birth or during the postnatal period. The definition of the German MAK Commission, as well as many other definitions, is very similar and includes any alteration from the physiological norm in the development of the organism which leads to pre- or postnatal death or to permanent morphological or functional damage of the offspring.

The question of how to classify developmental toxicants is closely associated with the methods by which they are detected.

Recognition of developmental toxicants is possible through experimental animal studies, epidemiological observations, or specific identification of malformation syndromes. All have a number of pitfalls and drawbacks that need to be addressed but cannot be dealt with at length in this review.

Animal studies

No animal studies have led directly to identification of a human developmental toxicant (Shepard 1982). However, when data for many of the agents recognized as human developmental toxicants have been compared to the experimental animal data, in almost all cases the agents have been found to produce developmental toxicity in animals, too. In at least one species tested the types of effects were similar to those in humans (Nisbeth and Karch 1983; Kimmel et al. 1990; Slikker 1994). Therefore it is widely accepted that there is ample evidence that effects produced in animal models are predictive of human outcome for basically all kinds of agents. As to the degree of certainty of predicting that an effect seen in animals could occur in humans, there are a number of confounding differences between mammalian species. These differences in both structure and physiology account for the fact that concordance between animal and human data bases is limited. In particular, concordance is not sufficiently high that the effects observed in animals would serve as guidance for clinicians and epidemiologists to focus only on certain types of adverse outcomes of pregnancy (Schwetz 1994). This lack of concordance must not be lamented, because for the detection and classification of developmental toxicants it is not the type of damage but any damage that matters. Therefore, the U.S. EPA (1991) concluded that a biologically significant increase in any of the four manifestations of developmental toxicity (death, structural abnormalities, growth alterations, and functional deficits) may be considered indicative of an agent's potential for disrupting development and producing a developmental hazard. Similarly, Moore et al. (1995), discussing cross-species extrapolation, consider it to be one of several default assumptions "that any manifestation of reproductive or developmental toxicity is relevant to humans unless the mechanism by which it occurs is impossible in humans."

The importance of the type of damage notwithstanding, the great value of animal studies with respect to developmental toxicity is that they provide the only means of establishing dose-response relationships which are central to the understanding of developmental toxicity and without which quantitative evaluation is not possible (O'Flaherty and Clarke 1994).

Well-defined regulatory test guidelines for developmental toxicity are available, the most recent being the ICH harmonized tripartite guideline on "detection of toxicity to reproduction for medicinal products" (ICH 1994), which is also applicable to chemicals. A fairly recent survey on and description of available testing procedures, including a test for determining the priority of substances for further investigation, was provided by ECETOC (1992). The studies defined in the guidelines should be considered the default protocols that are used when there is no other protocol that is known to be more appropriate for the chemical under evaluation (Schwetz 1994). It needs to be emphasized, however, that despite their proven utility, animal data can be fallible, thereby necessitating that the review and interpretation be performed by scientists with appropriate training and experience (Moore et al. 1995).

Human studies

Literature addressing such studies is numerous. With regard to the present question as to the utility as well as the limitations of human studies for detecting developmental toxicants, reference will be made only to review articles.

According to a 1985 OTA Report (U.S. Congress 1985), epidemiological studies can be divided into three broad classes: descriptive, analytical, and experimental.

For ethical reasons, experimental studies are difficult to undertake in industrial settings because subjects must be assigned to exposed groups. Therefore, such studies are practically nonexistent and only descriptive and analytical studies are utilized for studying reproductive impairment.

Case reports and large-scale surveillance programs are the two types of descriptive studies. Case reports have been more successful so far than surveillance programs. For example, DBCP infertility and rubella as a causative agent of birth defects were detected in case reports.

Analytical studies are subdivided into cross-sectional, case control, and cohort studies. The significance of these studies for risk assessment in prenatal toxicity has recently been discussed by the U.S. EPA (1991), Goujard (1992), ECETOC (1992), EEC (1993 b), and Moore et al. (1995), and, somewhat earlier, by Levin (1983).

A cross-sectional study, in which a group of people is surveyed for risk factors (exposure) and disease, does not

establish when exposure happened in relation to the development of disease. Therefore, such studies cannot establish cause and effect. In a case-control study, which is always retrospective by definition, the frequency of exposure to a potentially toxic substance in a group of affected individuals is compared to the frequency of exposure in a control group representing the underlying distribution in the study base. A cohort study is a prospective study in which two groups of people with different levels of exposure are followed up and effects are recorded over time.

The major reasons rendering human studies mostly inappropriate for the detection of developmental toxicants are twofold: *First*, reproductive toxicants only very rarely produce clearly visible effects (such as the thalidomide malformations) that are likely to establish an association with an exposure. Because developmental toxicity is mostly subtle rather than overt, the epidemiological studies tend to lack strength of association and consistency of observations and are compromised by confounding. It is, therefore, not surprising that it took more than half a century to delineate from early incidental reports the now well-defined fetal alcohol syndrome consisting of craniofacial, limb, and cardiovascular defects (Jones and Smith 1975). *Second,* and above all, the majority of published studies do not provide any evidence of dose-response due to lack of relevant exposure data, which explains why dose-response evaluation is usually based on the assessment of data from studies performed in animal species.

Summarizing the ways and means of detecting developmental toxicants, the advantage of properly planned state of the art animal studies over epidemiological observations is paramount, particularly with respect to occupational and preventive medicine. This is true despite the pitfalls inherent in animal studies. Hopefully, mechanistically based markers of developmental toxicity (Schwetz 1994) will soon be found which will then increase the predictive value of this kind of study.

Fundamentals of classification of potential developmental toxicants

Karnofsky (1965) first stated the concept now established as Karnofsky's law: Virtually any substance is capable of adversely affecting the conceptus if given at a high enough dose level. Following Hart et al. (1988), this law implies that any chemical is capable of causing adverse prenatal effects not only when given at the proper dose, but also by the proper route during the proper period of gestation to the proper species. This law, along with the conceptus's potentially unknown presence in the workplace and the fact that agents may cause damage to the conceptus at concentrations that have no adverse effect on its mother, gives rise to much concern. Classification of potential developmental toxicants is likely to at least reduce this concern. Classification is fundamentally possible because there is consensus that first, a threshold is assumed for the dose-response curve for developmental toxicants and that second, a "no observed adverse effect level" (NOAEL) or a benchmark dose can be determined or can be calculated as a basis for quantitative risk assessment.

The threshold assumption

It is assumed that there is a threshold for the chemical induction of nonheritable developmental effects as for other types of toxicity, except carcinogenicity. The assumption, now undisputed among developmental biologists, is "based on the hypothesis that there is a range of exposures from zero to some finite level that can be tolerated with essentially no effect. In this dose range, homeostatic or compensatory mechanisms are assumed to be present that can maintain the system until an exposure level, the threshold, is reached above which adverse effects will result" (Kimmel 1990). According to a very similar explanation (U.S. EPA 1991), the assumption is "based on the known capacity of the developing organism to compensate for or to repair a certain amount of damage at the cellular, tissue, or organ level."

The assumption, of course, is also accepted by the Commission of the European Communities, which states that reproductive toxicity, which by definition includes developmental toxicity, "is usually considered to be an effect with an underlying dose threshold mechanism" (EEC 1994).

The practical implication of the threshold assumption is that an agent that has produced adverse effects under certain experimental conditions must not be a hazard at every exposure level or in every situation. A classification scheme that would meet the needs of the situation at workplaces should clearly separate developmental toxicants that are likely to be hazardous from those that are not. This can be achieved by use of what has been described in many publications as the NOAEL/uncertainty factor approach (Kimmel 1990; U.S. EPA 1991; Kimmel and Kimmel 1994; Moore et al. 1995).

The NOAEL/uncertainty factor approach

The NOAEL/uncertainty factor approach depends on the choice of a critical effect (for example fetal weight) produced by the lowest dose level from a group of studies on the agent under consideration, and the choice of an appropriate NOAEL based on this critical effect. The NOAEL, as defined by the U.S. EPA (1991), is "the highest dose at which there is no statistically or biologically significant increase in the frequency of an adverse effect in any of the possible manifestations of developmental toxicity when compared with the appropriate control group in a data base characterized as having sufficient evidence for use in a risk assessment." To arrive at the reference dose (RfD) or reference concentration (RfC) for developmental toxicity (RfC sub DT), which is an estimate of a daily exposure of humans that is assumed to be without appreciable risk of deleterious developmental effects (U.S. EPA 1991), the

NOAEL is divided by the product of uncertainty factors (UFs). The UFs are intended to reflect interspecies differences, intraspecies variability, quality and quantity of data, pharmacokinetics, slope of the dose-response curve, and other factors. The total size of the UF varies and is subject to considerable controversy (Renwick 1991). Moore et al. (1995) state that, unless it has been modified by some other factor of uncertainty, the UF applied to the NOAEL is generally 100. Illing (1991) discussed the practice in the UK and stated that the UF will generally be 5–100 with 10 as the most common value. Hogan and Hoel (1982) have stated that there is no biological justification for routine use of any given safety factor, including the generally adopted 100.

The benchmark dose approach

There are clearly limitations and criticisms of the NOAEL approach (Kavlock and Kimmel 1992; Kimmel and Kimmel 1994; Moore et al. 1995), the most important of which is the fact that the NOAEL is limited to one of several experimental dose groups and is therefore dependent on the number and spacing of the dose groups, which in many studies is arbitrary rather than reasonably justified. Since use of the NOAEL focuses only on that one dose which is the NOAEL in a particular study, all other information on the slope and variability of the dose-response relationship is ignored. The benchmark dose (BD) approach, originally proposed by Crump (1984), involves statistical modeling using all the data of the experimental range to calculate a BD at which the chemical produces a small but measurable increase in the frequency or severity of its effect. Hence the BD is a model-derived estimate of a particular incidence level, such as to give a 5% or 1% extra risk over the background and its 95% lower confidence limit. Using the BD dose approach, values for each effect indicating embryo-fetal or maternal toxicity of a chemical can be calculated for which sufficient data are available. Unlike for drugs, sufficient data for many occupational substances are not available. Auton (1994) has reported recently on the calculation of BDs from 154 dose-response teratology data sets (compounds or classes of compounds not named) using the Weibull dose-response model, which gave an adequate fit to most data sets. He found the BD to be a convenient measure of potency of the chemicals in the animal bioassay, which together with an appropriate uncertainty factor "ensure that humans are adequately protected in the face of acknowledged uncertainties in extrapolating the bioassay results to low doses and across species." Details of the current discussion relating to the BD approach – specifically to the characterization of data bases as either quantal or continuous, to the determination of NOAELs, and to the statistical models appropriate for representing the unique features of developmental toxicity testing – will be found in the most recent comprehensive publications by Barnes et al. (1994), Faustman et al. (1994), and Allen et al. (1994 a, b). These authors have shown that the BD is applicable to existing data and that, with few exceptions, the BD results are generally in agreement with NOAELs determined by traditional methods.

Based on the threshold assumption and the NOAEL/UF approach the German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area ("MAK Commission") has introduced its classification concept, which is described and exemplified below.

Classification concept of the MAK Commission

Maximum allowable concentrations of chemicals at workplaces, such as the German MAK values, the US ACGIH-TLVs, the US OSHA-PELs, or the UK HSE MELs or OESs, are established for healthy persons of working age. While these exposure limits are established using the NOAEL/UF approach, the UFs actually applied for a given chemical are rarely, if ever, published. This is apparently due to the fact that establishing these limits is usually a consensus process performed in a case-specific manner.

Are these exposure limits, which are widely used in many countries and which do not generally differ significantly from each other, also applicable for healthy women of childbearing age who usually do not know they are pregnant during organogenesis (18th through 60th day of gestation)? The answer is clearly no for the majority of chemicals for at least two reasons. First, in many lists of occupational standards, the issue is not even addressed and second, there are a great number of chemicals with exposure limit values for which the potential of inducing developmental toxicity has been studied not at all or insufficiently. The only list that provides relevant information as to the applicability of the listed values for a given chemical is the German list of maximum concentrations (MAK) and biological tolerance values (BAT) (DFG 1994 a). In this list, all chemicals for which MAK and BAT values are established are (or will be) classified according to a quantitatively based concept (Hoffmann et al. 1983) which became effective in 1985 and was modified in the following years (Hoffmann et al. 1988; Hoffmann 1991).

Group A

Classification criterion: A risk of damage to the developing embryo or fetus has been unequivocally demonstrated. Exposure of pregnant women can lead to damage to the developing organism even when MAK and BAT values are observed.

The only chemical so far classified as belonging to group A is *methyl mercury.* The classification is a precautionary measure because there is sufficient evidence that the chemical is a potent human teratogen for which appropriate exposure data, however, are not available.

Group B

Classification criterion: Currently available information indicates that a risk of damage to the developing embryo or fetus must be considered to be probable when pregnant women are exposed, even when MAK and BAT values are observed.

Prominent examples among 16 chemicals currently classified as group B (seven of which are glycol ethers) are carbon disulfide (CS_2) and dimethylformamide (DMF).

Based on state of the art multispecies animal data, CS_2 is very unlikely to be a developmental toxicant at or below 10 ppm, but there are conflicting reports about diverse malformations observed in children of CS_2-exposed mothers. These reports call for caution although they neither establish a causal relationship nor provide reliable information on dose-effect relationships. Unlike methyl mercury, CS_2 was not classified as a group A chemical because the animal data for CS_2 justify doubts as to its potency as a developmental toxicant (DFG 1992).

DMF exemplifies a line of argumentation very different from the previous one: complete lack of human data relative to developmental toxicity, but conclusive evidence of high embryotoxic and teratogenic potential in three animal species after various routes of exposure, including inhalation. The "no observed adverse effect concentrations" (NOAECs) (inhalation) were found to be 50 ppm for rabbits and 18 ppm for rats, with "low observed adverse effect concentrations" (LOAECs) between 50 and 150 ppm. Better spacing of the concentrations probably would have resulted in higher NOAECs but since this is speculative, it was decided that the difference between the MAK of 20 ppm and the NOAECs in animal studies was low and that the risk of developmental toxicity would be substantial even if 20 ppm were not to be exceeded. In addition to inhalation of the chemical, dermal absorption had to be taken into account. The lack of a minimal UF of 10 and dermal absorption as a serious confounding factor called for classification of DMF as belonging to group B (DFG 1992).

Group C

Classification criterion: There is no reason to fear a risk of damage to the developing embryo or fetus when MAK and BAT values are observed.

Chemicals classified as group C represent a very heterogeneous group of substances ranging from (local) irritants to those producing systemic toxicity after absorption and distribution to the target at a site distant from the entry point. Currently 54 chemicals are listed as belonging to group C, including several that can be found in lists of teratogens, such as ethanol, toluene, and dimethyl acetamide, to name but a few. These three chemicals will be discussed in some detail to illustrate the quantitative aspects of the classification scheme.

Although it has been recognized since antiquity (Schardein and Keller 1989), the association between congenital malformations and alcohol consumption by pregnant women was first published only in 1967. Today, fetal alcohol syndrome (FAS) is a well-known expression of distinct developmental toxicity but it remained doubtful until recently whether exposure to 1900 mg/m^3 (MAK and also TLV) *ethanol* was likely to be toxic to the unborn child. Therefore, a study was initiated by the Commission in which 24 healthy young volunteers (12 men, 12 women) were exposed for 4 h to different ethanol concentrations up to 1500 mg/m^3. The average blood ethanol concentration resulting from exposure to 1500 mg/m^3 was 2.18 mg/l (0.00218‰). Blood ethanol concentrations were linearly related to alveolar air and ambient air concentrations, and no significant sex-specific differences were noted (Golka et al. 1994). It is generally accepted today that first signs of subtle developmental toxicity do not appear below maternal blood concentrations of 0.01‰ (DFG 1994b), while maximum blood ethanol levels of about 200 mg/100 ml (2.5‰) in mice and 25–50 mg/100 ml (0.25‰–0.5‰) in rats and rabbits have been reported to have caused maternal toxicity, but no embryotoxic and no teratogenic effects (Schwetz et al. 1978). Since the average blood level of 0.002‰ resulting from exposure to 1500 mg/m^3 ethanol is one order of magnitude lower than the above-mentioned 0.01‰ and very much lower than the apparent threshold in animals, ethanol was classified as belonging to group C. The Commission thus has shown that listing of ethanol as a human teratogen does not make sense when viewed in a workplace context.

Toluene is known to be capable of producing a syndrome of malformations and dysfunctions similar to FAS after very high exposures following occupational or chronic substance abuse (sniffing) (Schardein and Keller 1989). Because of uncertainties concerning the NOAECs in animal studies, the chemical was originally classified B, but when additional rat studies became known and new rabbit studies, initiated by the Commission, were available, it was reevaluated and classified C in 1993, when the MAK value was reduced from 100 to 50 ppm (which is also the TLV). The reasoning for C was conclusive: human developmental toxicity has only been associated with abusive exposures and the NOAECs determined in state of the art inhalation studies were 400 ppm for mice, 750 ppm for rats, and 500 ppm for rabbits. These NOAECs were tenfold higher than the MAK (and TLV) value of 50 ppm; in view of the metabolic and kinetic similarities between humans and animals, the margin of safety was judged adequate to conclude that exposure to 50 ppm does not impose a risk of developmental toxicity (DFG 1993).

Unlike with ethanol and toluene, there are no reports on adverse effects on children after maternal exposure to *N,N-dimethyl acetamide* (DMAc). High doses/concentrations of this widely used solvent can produce embryotoxicity and also teratogenicity in laboratory animals after different routes of application; however, the dose-effect relationships for this chemical are well characterized and NOAELs/NOAECs have been established not only for oral dosing but also in state of the art rat and rabbit inhalation studies. The NOAECs were found to be 100 ppm

for the rat (281 ppm was only marginally fetotoxic) and 200 ppm for the rabbit. The current MAK (and TLV) value of 10 ppm therefore gives a ten- to twentyfold safety margin over these NOEACs, which was judged adequate for classification of DMAc as belonging to group C (DFG 1990). This judgment is shared by the UK Health and Safety Executive, who noted, however, that concomitant absorption of DMAc via the skin should be avoided (Fairhurst et al. 1992).

Summarizing this section on classification into group C, it may be stated that, when dose-effect relationships are documented and are seriously considered, a large number of chemicals listed as developmental toxicants or teratogens can be classified according to their potency rather than their potential and may be handled safely at properly controlled workplaces.

Group D

Classification criterion: Classification into one of the groups A–C is no yet possible because while the available data may indicate a trend, they are not sufficient for a final evaluation. For each of these substances it is indicated which further studies are considered necessary to achieve definitive classification.

It comes as no surprise to people familiar with reproductive and developmental toxicity that the number of chemicals having a poor data set is very high. Currently 40 chemicals are listed under group D. The majority of these do not appear to impose a developmental toxicity risk at or below the allowable workplace concentrations. Interested parties are encouraged to initiate the studies necessary to bridge the gaps, as has been done for ethanol, toluene and others.

Examples of group D chemicals are nitrous oxide (N_2O), methanol, and ethyl benzene.

Reports on adverse reproductive effects of N_2O observed in assistants in operating theaters are manifold, as are data indicating embryotoxicity and teratogenicity of very high concentrations in animals. A definitive quantitative evaluation is currently not possible because of a lack of inhalation studies of concentrations in the order of the MAK value of 1000 ppm (DFG 1993).

The situation for *methanol* is different because of the substantial quantitative differences in the way rodents and primates metabolize methanol and formate. When methanol was classified D in 1989, it was largely assumed that the formate metabolite was responsible for the rodent teratogenicity (DFG 1989). In the meantime, research on unresolved aspects of methanol toxicity has been conducted, and indicates that the parent compound rather than the formate metabolite accounts for the developmental toxicity observed in rodents (Medinsky and Dorman 1994). These new findings necessitate reevaluation of methanol and are likely to result in its classification as belonging to group C.

Turning to the final example, *ethyl benzene* at 100 ppm (MAK and TLV) is very unlikely to be a developmental toxicant. But, because of controversial rodent data, it has to be classified D until the inconsistencies in the data set are resolved (DFG 1992).

Unclassified chemicals

Finally, there are currently 65 chemicals listed for which no data at all are available. These chemicals cannot be classified by whatever scheme. They may be subject to speculation and to structure-activity considerations which, however, are deemed totally inappropriate for exposure assessment because structure-activity relationships have not been well studied in developmental toxicity (U.S. EPA 1991). There may be certain "structural alerts" by which a chemical of interest can be likened to a chemical known to be toxic to reproduction (EEC 1994), but a science-based judgment is not feasible unless the substances are adequately tested. This does not necessarily mean that large numbers of animals need be used in the first place. The pilot study widely used in industry to select the dose levels for standard embryotoxicity studies requires only a limited number of animals and nevertheless provides valuable information. This information will not qualify a substance eligible for classification on a quantitative basis, but it will at least provide guidance where to place the substance in question on a priority list for further testing. This approach was adopted and pursued by OECD experts in 1990 and has resulted in guideline 421 describing in detail a reproduction/developmental toxicity screening test (OECD 1994).

While a number of existing chemicals still need to be adequately tested, some of the unclassified chemicals might be classified without having been tested, because lack of developmental toxicity can be reasonably concluded on theoretical grounds. To conclude this review, one such example is briefly discussed. *Phosphorus pentoxide* was classified C because, when inhaled, it is completely hydrolyzed to orthophosporic acid. The resulting phosphates are physiological substances. The amount of phosphates formed after 8-h inhalation of 1 mg/m^3 (the current MAK value) is much too low to significantly affect the blood pH or the overall body phosphate balance. Therefore, developmental toxicity can be excluded (DFG 1994b).

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