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A regulatory approach to assess the potency of substances toxic to the reproduction

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ABSTRACT

In order to develop a method for setting specific concentration limits (SCLs) for substances toxic to the reproduction within the European classification and labelling system, this study investigated possible parameters for reproductive toxicity potency and the quantitative distribution of those parameters. For that purpose, two databases were created comprising substances classified in the European Union for developmental toxicity or for effects on sexual function and fertility. For these substances six parameters including NOAEL, LOAEL and ED_{10} were determined for effects on reproduction based on existing data summaries. The potency was defined independent of the type of reproductive effect as generally severe effects on reproduction warranting classification were already observed at the lowest dose showing reproductive effects. The reproductive toxicity potency range of substances in the databases was a factor of approximately one million. This shows that SCL setting is needed to adjust the classification of mixtures. The average potency distribution of substances classified according to the hazard classification as required by the European CLP regulation in category 1 versus category 2 was similar. The ED_{10} for effects warranting classification is proposed as the best parameter for the potency based on its independence of administered dose levels.

1. Introduction

Classification and labelling of chemicals is an important instrument in the risk reduction management of chemicals. A Globally Harmonised System of Classification and Labelling of Chemicals (GHS) was recently developed by the United Nations (2009). This system was used as the basis for the development of legislation in several regions in the world including the Regulation on Classification, Labelling and Packaging of substances and mixtures (CLP) of the European Union (2008). GHS and CLP allow the determination of the hazards of a substance using a number of criteria. The classification of mixtures for all health hazards except acute toxicity and the aspiration hazard is determined by the classification of the ingredients and their percentage in the mixture. For these health hazards, generic concentration limits (GCLs) have been set above which the classification of a substance present in a mixture will result in the classification of the mixture for that hazard. These GCLs may be adequate for most substances but may under- or overestimate the hazard of a mixture depending on the toxic potency of the substance under consideration. This is especially true for hazards generally classified on the basis of a weight of evidence approach but independent of the potency such as carcinogenicity, mutagenicity and reproductive toxicity. The GHS and the CLP therefore allow the determination of deviating specific concentration limits (SCLs). Substances which are expected to show that hazard at concentrations below the GCL are assigned a lower SCL. In exceptional circumstances higher SCLs are assigned for substances which do not show that hazard at concentrations above the GCL. However, neither precise methods nor guidance for the determination of SCLs for each specific hazard are described in the GHS and CLP. According to article 10 part 7 of CLP, guidance will be provided by the European Chemicals Agency (ECHA) for the

Abbreviations: GHS, globally harmonised system of classification and labelling of chemicals; CLP, regulation on classification labelling and packaging of substances and mixtures; GCL, generic concentration limit; SCL, specific concentration limit; LOAEL, lowest observed adverse effect level; NOAEL, no observed adverse effect level; ED_{10}, effective dose with a 10% effect level above the background.

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determination of SCLs. A method for the determination of SCLs for carcinogenicity has already been developed in the EU under the Directive EC 67/548 (European Commission, 1999), which also applies to the CLP regulation. For the hazard classes mutagenicity and reproductive toxicity, no analogous methods are available yet. This study investigated the possible parameters for the estimation of toxic potency for the hazards developmental toxicity and adverse effects on sexual function and fertility and the distribution of those parameters. For that purpose, two databases were created comprising substances classified within the European Union for developmental toxicity or for effects on sexual function and fertility. This information was needed for the development of a guidance method for the determination of SCLs for reproductive toxicity.

Regulation (EC) No 1272/2008 (CLP) and GHS contain rules for the classification of substances and mixtures. In Chapter 3.7 of Annex I to the CLP Regulation, criteria are given for the classification of substances as reproductive toxicants in category 1 for known or presumed human reproductive toxicants and in category 2 for suspected human reproductive toxicant. Substances are classified in category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (category 1A) or from animal data (category 1B). Substances are classified in category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in category 1. If deficiencies in the study make the quality of evidence less convincing, category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects. The classification as reproductive toxicant is independent of the potency.

The potency of a substance within classification and labelling can be expressed as the dose at which a certain adverse effect is observed in a certain incidence and/or magnitude. Examples are the LD_{50} for acute toxicity, the EC3 value for skin sensitisation (Basketter et al., 2005) and the T25 for carcinogenicity (Sanner et al., 2001). For other health hazard classes there may be several different types of effects which define the potency. This includes specific target organ toxicity after single and repeated exposure. For these hazard classes, a number of different specific effects (e.g. hematotoxicity or nephrotoxicity) are included in the classification criteria which will result in classification or not. The dose level at which one or more of these effects is observed determines the potency. The potency of substances classified for reproductive toxicity can be based on the dose level at which a certain effect is observed at a certain level. The criteria contain a number of adverse effects for both developmental effects and effects on sexual function and fertility warranting classification. However, it can be questioned whether all these adverse effects can be assigned with the same severity. For example a reduction in the male gamete production could be considered as less severe compared to a reduction in the number of offspring. Both these effects could be observed in one study at different dose levels. This makes it difficult to define one level of comparable effect severity. Therefore, several possible estimates for potency were investigated in the present analysis. Further, the study design in terms of species and strain, exposure route, exposure duration, exposure window in the life cycle, and possible concomitant parental toxicity could affect the dose level at which certain effects are observed. A database to derive potency estimates for reproductive toxicity was lacking and had to be established. Therefore, data on substances classified for developmental toxicity or on the impairment of sexual function and fertility were collected. The collected parameters included the NOAEL, LOAEL and ED_{10} (effective dose with a 10% effect level above the background) for effects on development or sexual function and fertility of each substance. NOAEL and LOAEL were added in the analysis because these parameters are widely used in risk assessment in Europe. The BMD_{10} is considered a more accurate estimate of the dose with an effect size of 10% than the ED_{10} because it takes into account all the available dose effect information from the study. However, due to the limited resources it was not possible to determine the BMD_{10} for all the substances. Also other data such as whether a substance is classified for mutagenicity, the type of effect at the LOAEL and species used in the test were taken into account. These parameters were analysed for an effect on the potency of the substances.

2. Method

Substances classified by the European Union for developmental toxicity (CLP categories 1A, 1B or 2) or for effects on sexual function and fertility (CLP categories 1A, 1B or 2) were selected from the ex-ECB Classlab database using both the Annex I database and the working database (ex-ECB, 2008). This means that substances which were already included in Annex VI of CLP and substances that were concluded by an European advisory working group but that were then not yet included in Annex VI of CLP were considered in the analysis. Group entries were excluded. For the remaining substances, NOAEL, LOAEL and ED_{10} for developmental effects or effects on sexual function and fertility were determined. These parameters were determined either for effects fulfilling the European criteria for classification or for any effect on development, sexual function or fertility. Maternal or systemic effects were not taken into account when deriving these parameters because these effects are already taken into consideration during the classification and should not be used again to influence SCL setting. These parameters will be named NOAEL classification, LOAEL classification and ED_{10} classification or NOAEL overall, LOAEL overall and ED_{10} overall. The ED_{10} was determined by linear interpolation where possible using the effect with the highest incidence or magnitude at the LOAEL. The approach is different depending on whether the effect is measured as an incidence (quantal data) or a magnitude (continuous data). For effects based on incidence, like the percentage of malformations, the ED_{10} was determined as 10% above the incidence in the control group. This means an increase for example from 2% in the control group to 12% for the ED_{10}. For effects based on magnitude, like foetal weight, the ED_{10} was determined as 10% difference with the control group. The following information was also included in the database: the name and CAS number of the substance, the type of study determining the LOAEL overall and LOAEL classification, the species determinative for the LOAEL overall and LOAEL classification, the route of exposure determinative for the LOAEL overall and LOAEL classification and the classification for mutagenicity and reproductive toxicity. The type of developmental effects at the LOAEL was determined and divided into four classes namely malformations, foetoletality, functional changes (neurotoxicity and behavioural changes) and others. The type of effects on sexual function and fertility at the LOAEL was determined and divided into five classes namely reduced fertility, adverse effects on sperm, weight of reproductive organs, histopathological changes of reproductive organs and other effects.