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Assessment of reproductive and developmental effects of DINP, DnHP and DCHP using quantitative weight of evidence

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ABSTRACT

Quantitative weight of evidence (QWoE) methodology utilizes detailed scoring sheets to assess the quality/reliability of each publication on toxicity of a chemical and gives numerical scores for quality and observed toxicity. This QWoE-methodology was applied to the reproductive toxicity data on diisononylphthalate (DINP), di-n-hexylphthalate (DnHP), and dicyclohexylphthalate (DCHP) to determine if the scientific evidence for adverse effects meets the requirements for classification as reproductive toxicants. The scores for DINP were compared to those when applying the methodology DCHP and DnHP that have harmonized classifications. Based on the quality/reliability scores, application of the QWoE shows that the three databases are of similar quality; but effect scores differ widely. Application of QWoE to DINP studies resulted in an overall score well below the benchmark required to trigger classification. For DCHP, the QWoE also results in low scores. The high scores from the application of the QWoE methodology to the toxicological data for DnHP represent clear evidence for adverse effects and justify a classification of DnHP as category 1B for both development and fertility. The conclusions on classification based on the QWoE are well supported using a narrative assessment of consistency and biological plausibility.

1. Introduction

The process of hazard assessment and risk characterization should include a science-based evaluation of all of the available data on the investigation of the toxicity of a specific chemical (Beronius et al., 2014; Lutter et al., 2015; Rhomberg, 2015; Schreider et al., 2010; US-EPA, 2005). Traditionally, hazard assessments and risk characterization have relied on scientific judgment with a narrative assessment and have often only included results from "key studies". Consequently, the process has been criticized for lack of objectivity and transparency (see for example, Myers et al., 2009). However, conclusions based on the overall toxicity database often require integration of several lines of evidence (different types of studies) with different research objectives, applied methodologies and study quality. The available database may include peer-reviewed publications often addressing selected endpoints with potential relevance to toxicity, but also reports on the results of targeted toxicity testing following specific protocols required by legislation. In addition, the relevance of effects reported in scientific publications may be controversial. Therefore, narrative assessments have a number of weaknesses. To improve their quality, weight of evidence (WoE) approaches are increasingly mandated in chemical regulations (Agerstrand et al., 2014; ECHA, 2015; Weed, 2005). However, detailed guidance to perform WoE assessments is lacking and quantitative aspects only received limited considerations (Rhomberg, 2015; Van Der Kraak et al., 2014).

A recently developed quantitative weight of evidence (QWoE) approach to assess toxicity data for chemicals is designed to assist with classification and labeling (C&L) regarding reproductive toxicity endpoints (Dekant and Bridges, 2016). This QWoE applies predefined scoring criteria for relevant aspects of quality/reliability of a study for all reported effects to provide a fully transparent assessment. The scores representing strength of evidence for adverse effects are then compared to benchmark scores that are anchored to adverse biological endpoints and serve as the basic requirements for classification.

This QWoE was used to assess a need for C&L regarding findings from reproductive toxicity studies of three phthalates, diisononlyphthalate (DINP), dicyclohexylphthalate (DCHP), and di-n-hexylphthalate (DnHP). Phthalates are widely used as plasticizers.

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Glossary of terms

Weight of evidence method (WoE)	The identification and objective analysis (using predefined, scientifically justified criteria) of all potentially relevant studies, for their quality and in testing a hypothesis (problem formulation)
Quantitative weight of evidence (QWoE)	The identification, objective analysis and numerical scoring (using predefined scientifically justified criteria) of all potentially relevant studies, for both their quality and relevance in testing a hypothesis (problem formulation)
The hypothesis	Generally, takes the form of "does chemical of interest X cause adverse effects Y under conditions Z". Conditions may include exposure levels and duration, species of interest, adverse effects are defined as by WHO/IPCS
Endpoints	The measured and modelled findings used to identify and characterise adverse effects Y
Quality	The reliance that can be placed on the findings of each study for the purpose of critically testing the hypothesis
Relevance	The utility of the findings of each study on adverse endpoints for the purpose of critically testing the hypothesis
Lines of evidence	The different types of investigation used to critically test the hypothesis (e.g. observations in man, targeted toxicity testing in animals, in vitro experiments determining molecular endpoints, and in silico predictions of toxicity based on read-across or quantitative structure activity relationships)
Weighting of endpoints	A multiplier that is applied to the relevance/effect scores to reflect the relative importance of different types of endpoint and/or different lines of evidence in support of the hypothesis
Strength of evidence	This score is derived by multiplying the final relevance/effects scores by the quality/reliability score for a particular study
Overall weight of evidence	This is a summation of the findings from all suitable studies. It may be presented graphically as a plot of relevance/effects against quality scores or as an average numerical value with ranges

Di(2-ethylhexyl)phthalate (DEHP) was a plasticizer of choice, but has been replaced by higher molecular weight phthalates such as DINP in many applications due to concerns regarding possible adverse effects of DEHP-exposures in humans. A concern for phthalates in general, with a focus regarding adverse reproductive and developmental effects, has been raised based on results from animal toxicity studies with certain low molecular weight phthalates. In rats, application of high doses of di-n-butylphthalate (DBP) and DEHP during specific phases of pregnancy induce reproductive toxicity in male offspring (EFSA, 2005a; EFSA, 2005b; EFSA, 2005c; EFSA, 2005d; EFSA, 2005e). The effects include malformations of the epididymis, vas deferens, seminal vesicles, prostate, external genitalia (hypospadias) and cryptorchidism, as well as retention of nipples/areola (sexually dimorphic structure in rodents) and demasculinization of the perineum resulting in a reduction in anogenital distance (AGD). This pattern is sometimes termed the "phthalate syndrome" and is speculated as similar to a human disease termed 'testicular dysgenesis syndrome' (TDS). TDS in humans is hypothesized (Juul et al., 2014; Main et al., 2010; Sharpe and Skakkebaek, 2008) to account for many common disorders of newborn (such as cryptorchidism and hypospadias) and young adult males (such as low sperm count and testicular germ cell cancers) but the mode of action and underpinnings of TDS are unclear,. This concern on phthalates has raised a discussion on safety regarding many applications and resulted in national strategies regarding replacement of phthalates in commerce. However, the reproductive toxicity of low to medium molecular weight phthalates is different with clear effects observed for DEHP, DBP, and di(isobutyl)phthalate (DiBP) in one- and/or multigeneration studies (EFSA, 2005a; EFSA, 2005b; EFSA, 2005c; EFSA, 2005d; EFSA, 2005e; EU-RAR, 2008) whereas high molecular weight phthalates such as diisononylphthalate (DINP) and diisodecylphthalate (DIDP) did not induce such effects and reproductive toxicity is not considered a concern with dimethyl (DMP) and diethylphthalate (DEP) (Anonymous, 1997; Field et al., 1993; Gray et al., 2000; Hushka et al., 2001; SCCP, 2007; Waterman et al., 1999, 2000).

The purpose of the application of a QWOE to the toxicity database on DINP, DCHP, and DnHP was to assess the robustness of the QWOE-methodology and the relevance of reported effects in the scientific literature in a transparent, consistent and scientifically justified way, using predetermined scores for quality and relevance/effects. DnHP and DCHP have harmonized classifications according to the CLP regulation as category 1B reproductive toxicants (DnHP for both development and fertility; DCHP only for development), while, according to the European Risk Assessment report (EU-RAR, 2003), a classification of DINP was not mandated at the time of the preparation of the EU-RAR. Since completion of the EU RAR, little new information on the effects of DINP on reproductive endpoints has been generated and is integrated here.

2. Methods

In the first step, potentially useful publications for assessment purposes on the animal toxicology of DINP, DCHP, and DnHP were searched with a cut-off date of July 31, 2015. To capture all publications and minimize search-bias, the literature search included PubMed, TOXLINE, Chemical Abstracts, and SciFinder with the following search terms:

- CAS # 84-61-7 (DCHP), CAS # 84-75-3 (DnHP), CAS # 28553-12-0, and CAS # 68515-48-0 (both DINPs) in "ToxLine",
- CAS # 84-61-7, CAS # 84-75-3, CAS # 28553-12-0, and CAS # 68515-48-0 and "toxicity" in "Chemical abstracts"
- CAS # 84-61-7, CAS # 84-75-3, CAS # 28553-12-0, and CAS # 68515-48-0 and "toxicity" in "PubMed"