Science of the Total Environment 435–436 (2012) 526–540

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Ecotoxicity of paint mixtures: Comparison between measured and calculated toxicity

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HIGHLIGHTS

- ► CA calculations of EL_{50Mix} were conservative for half of the mixtures tested.
- ► Most IA calculations of toxic effects resulted in less toxicity than was observed.
- ► The DPD+ approach in REACH led to conservative predictions for three of five mixtures.
- ► The curing process caused enhanced and decreased toxicity in different mixtures.
- ► Adaptations of test procedures and development of analytical methods were required.

ARTICLE INFO

Article history:
Received 30 March 2012
Received in revised form 4 July 2012
Accepted 4 July 2012
Available online 11 August 2012

Keywords:
Anticorrosive paint mixture
Aquatic ecotoxicity
Concentration addition
Independent action
REACH

ABSTRACT

The aims of the current study were to test the validity of the concentration addition (CA) and independent action (IA) calculation methods for mixture toxicity as well as the REACH guidelines for mixture exposure scenarios for paint products. Based on ecotoxicity tests of nine anticorrosive paint mixtures and the nine substances that contributed to classification of the mixtures as hazardous for the aquatic environment, neither CA nor IA was found to be appropriate calculation methods, although CA calculations were conservative for half of the mixtures tested. The REACH mixture approach, based on “lead” substances, resulted in conservative predictions for three out of five mixtures and the “lead” substance corresponded to the substance contributing the most in the CA mixture calculations for four out of six mixtures. The use of paints as a matrix for toxicity testing required adaptation of test procedures, development of sample handling and chemical analysis methods.

1. Introduction

Predicting mixture toxicity, based on information on the toxicity of single substances, is challenging and different methods have been tested, reviewed and discussed by scientists, governmental agencies and scientific organisations for almost a century (Backhaus et al., 2010; Kortenkamp et al., 2009). Most methods used to predict mixture toxicity were developed for substances that would not be expected to interact (Bliss, 1939; Kortenkamp et al., 2009; Könemann, 1981a), since interactive effects such as synergism and antagonism will render these predictive tools inappropriate for some substances (Bliss, 1939; Kortenkamp et al., 2009). The majority of toxicological tests and concepts developed to measure and predict mixture toxicity have not focused on commercial mixtures such as paints (Kortenkamp et al., 2009). Furthermore, the majority of ecotoxicological mixture studies that have been performed have tested binary mixtures (Bliss, 1939; Kortenkamp et al., 2009; Könemann, 1981a). Consequently, there is a need for more knowledge of the joint action of realistic commercial mixtures to substantiate current concepts describing the joint action of substances in mixtures (Kortenkamp et al., 2009). The comparison of mixture toxicity test data with predictions of joint action (i.e. concentration addition and independent action) for commercial anticorrosive paint products was explored in the current study.

Plackett and Hewlett's (1952) definitions of “simple similar joint action” as two substances eliciting a certain response by causing the same physiological system to react or fail and “dissimilar independent joint action” as two substances eliciting a certain response by different and distinct physiological systems were used in the current paper. The toxicity of mixtures with substances that are not synergistic in their effects can be calculated by concentration addition (CA) or independent action (IA) for substances that exhibit “simple similar joint action” and “dissimilar independent joint action”, respectively (Kortenkamp et al., 2009; Könemann, 1981a) and these calculation methods were used in the current study. CA has been suggested as a pragmatic and precautionary default assumption for mixture toxicity (Faust et al., 2003). The probability of independent action when the toxic effect occurs at an organism level has been suggested to decrease as the numbers of substances in the mixture increase, as many substances may

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doi:10.1016/j.scitotenv.2012.07.011

have some secondary effects in common but may have different primary toxic action (Deneer et al., 1988a). Syberg et al. (2008) concluded that CA and IA predict binary mixtures of similar and dissimilar toxicants equally well, but when applied to multi-cellular organisms it is presumably more likely that a toxicant will influence the toxicity of other substances.

The Regulation of the European Parliament and of the Council concerning REACH was adopted on 18 December 2006 (EC, EP, 2006) and entered into force on 1 June 2007. One of the overall goals of REACH was the "safe use" of chemicals so that they would not adversely affect human health or the environment (EC, EP, 2006, article 1(3)). The term "safe use" with regard to the environment under REACH was defined as use conditions that would give predicted environmental concentrations (PECs) below the predicted no effect concentrations (PNECs) (ECHA, 2008a). The manufacturer or importer of substances became ultimately responsible for the risk assessment, registration and development of exposure scenarios describing "safe use" and it is also their responsibility to pass such information to the downstream users of chemicals (EC, EP, 2006, article 31(7)). The responsibility for describing "safe use" of mixtures was, however, left to the formulators of mixtures. The treatment of mixtures, under REACH, is described in much less detail than the assessment of single substances (ECHA, 2007, 2008a,c,d). The guidelines for how a downstream formulator would be expected to develop mixture exposure scenarios are still under development (ECHA, 2008b,c, 2011g; CEFIC, 2009).

Anticorrosive paint systems generally consist of three anticorrosive paint products, a primer, a mid-layer and a top-coat, that are applied successively to prevent corrosion of steel surfaces with no intended release to the environment. Anticorrosive paint products are normally applied on surfaces above sea-level, although some anticorrosive paint products are used below sea-level. Each anticorrosive paint product comprises two components: component A and component B. Component A and component B are complex mixtures containing between 2 and 40 different substances and consist typically of chemical binders (usually polymers), pigments (organic or inorganic, may contain corrosion-inhibitors like zinc), extenders (mineral powders), volatile organic solvents and additives (e.g. paint driers, catalysts, antifoaming agents and dispersants). In anticorrosive epoxy paint products, component A contains an epoxy polymer, which will react upon mixing with the "hardener" of component B (usually a polyamine, polyamide, aminaduct or isocyanate) to give a rigid, three-dimensional, insoluble paint film that is resistant against chemicals, acids and alkalis (Ehlers et al., 2007; Herrick and Smith, 1987; Kjernsmo et al., 2000). The current study included ecotoxicity testing of 9 anticorrosive paint mixtures and 9 substances. The substances tested represented different chemical classes (e.g. metals, solvents, epoxies, amines). The tested aromatic hydrocarbons C8–C10, zinc powder (stabilized), zinc oxide, epoxy resin, fatty acid epoxy and dibutyltin dilaurate, all of which have water solubility values below 10 mg/L, were characterised as substances with very low solubility (Appendix B, Table B1), where adaptations of the prevailing toxicity test methods described in OECD guidelines 201 (OECD, 2011) and 202 (OECD, 2004) would be recommended (ISO, 2006; OECD, 2000). Xylene and ethyl benzene are "volatile" according to OECD (2000). For such substances, the loss due to evaporation may become significant under vigorous mixing conditions and the OECD guidelines 201 and 202 (OECD, 2004, 2011) have earlier been indicated not to be adequate (Girling et al., 1992). Consequently, adaptations to the toxicity test methods were introduced according to OECD (2000) and ISO (2006). These adaptations were also used for the mixtures that contained poorly water soluble and/or volatile substances. Water-accommodated fractions (WAFs) can be used to determine the toxicity of complex mixtures consisting of many substances that are only partly soluble in water (ISO, 2006; OECD, 2000). The water-accommodated fraction represents an aqueous media containing only the fractions of the substances that are dissolved and/or present as a stable dispersion or emulsion. Water-soluble fractions (WSFs) may be achieved by separating the un-dissolved product from the WAF (ISO, 2006; OECD, 2000). WSFs are advised to be used for metal compounds and mineral products (ISO, 2006). Since the mixtures of the current study contained metals and minerals, and each of the substances contained in the mixtures had different water solubilities, the mixtures were tested as WSFs (Girling et al., 1992). The WSF was also chosen instead of the WAF to avoid particles that may cause indirect effects in test organisms, particularly daphnids (Ruffli et al., 1998). The WSF was described as aqueous media containing only the fraction of a substance remaining in the aqueous phase once any source of mixing energy have been removed and after a period sufficient for phase separation and filtering (Girling, 1989). The resulting solutions were defined as "true solutions" and have been indicated by OECD (2000) as comparable to substance samples tested according to the general procedures (OECD, 2004, 2011).

The number of substances that were tested and included in the CA and IA calculations was low in relation to the total number of substances contained in the mixtures as only the substances that were taken into consideration when carrying out hazard classification (EC, EP, 1999) or making an exposure scenario for a mixture (EC, EP, 2006), were included. The hazard classification rules for mixtures excluded all substances that were not classified as dangerous for the aquatic environment, as well as substances in concentrations below 0.1% (of total weight) for substances classified as dangerous for the environment with the hazard symbol N. In addition, those substances below 1% (of total weight) for substances classified as "Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment", unless other specific limits were specified in the Annex V to the directive (EC, EP, 1999), were excluded (EC, EP, 1999, article 3(3)). Under REACH, a chemical safety assessment including an exposure scenario needs not be performed for a substance which is present in a mixture if the concentration of the substance in the mixture is less than the lowest of any of the following: the concentration limits given in the substance directive (EC, 1967); the concentration limits given in article 3(3) or the annexes to the preparation directive (EC, EP, 1999); the concentration limits established when a substance was registered; or 0.1% (of total weight), if the substance was classified as persistent, bio-accumulating and toxic, or very persistent and very bio-accumulating (EC, EP, 2006, article 14(2)). Xylene and ethyl benzene were also tested because these substances had been shown to be critical for environmental exposure in an earlier study (Gade et al., 2008). Excluding substances with different modes of action contained in non-toxic concentrations in the mixture would be in line with the IA approach (Boedeker et al., 1992). Even non-toxic concentrations of single substances would, however, be expected to contribute to the toxicity and should be included in CA calculations (Boedeker et al., 1992; Deneer et al., 1988b; Könemann, 1980). Defined sub-mixtures and response addition for the joint action of subgroups (Kortenkamp et al., 2009) or the two-stage prediction method (Qin et al., 2011) were not used because knowledge of the mode of action of many of the substances towards alga and daphnids was limited.

According to the European Chemicals Agency (ECHA, 2008c) three approaches may be used for the development of mixture exposure scenarios:

1. The Downstream User Guidance (ECHA, 2008b), by compiling relevant information of the substances of the mixture and selecting the appropriate set of risk management measures to control the risk for the mixture. Duplicates and inconsistencies shall be removed.
2. The rules of the Dangerous Preparation Directive (DPD) (EC, EP, 1999), by compiling the concentration and hazard classification of each substance of the mixture and apply the calculation rules of the DPD to identify the lead substance(s) driving the classification of the mixture. The risk management measures of these substances shall be used for the mixture, but additional risk management measures may need to be forwarded if other substances may present a risk.
3. The critical component(s) approach by using the substance PNECs and the concentration and other factors that may influence the