

Conference Paper

AQUATIC TOXICITY OF SELECTED CHEMICALS AS A BASIC CRITERION FOR ENVIRONMENTAL CLASSIFICATION*

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In order to protect public health and the environment, the EU legislation has proposed a classification of dangerous substances. Chemicals are classified according to physico-chemical as well as toxicological and ecotoxicological properties. Environmental classification is based on inherent harmful potential of a substance to organisms and on its environmental fate, that is, degradation and bioaccumulation potential. In this study, experimental data on acute and chronic toxicity to aquatic organisms and biodegradability and bioaccumulation data obtained from literature were used to classify arsenic (applied as As_2O_3), copper (applied as $CuCl_2$), phenol and 1,4 – butynediol. For this purpose, the "base set data" obtained from standardised test methods served as a convenient indicator of the inherent toxicity of tested chemicals. Additional data about environmentally relevant properties of arsenic and 1,4 – butynediol could lead to a revision of present chemical classification and labelling.

KEY WORDS: *aquatic environment, arsenic, 1,4-butynediol, copper, labelling of dangerous substances, phenol, ready biodegradability*

The aim of classification and labelling of dangerous substances is to protect the user, the public and the environment by indicating their harmful potential. The main purpose of environmental classification is to provide information on environmentally relevant properties of substances and clear advice to the user on how to avoid or minimise environmental exposure to dangerous substances (1).

The purpose of the first European Council directive 67/548/EEC was to harmonise the laws of the member states on the testing, classification, packaging and labelling of chemicals that are dangerous to people or the environment (2). The sixth amendment to the directive (3) introduced a pre-market testing and a notification system for new chemicals placed on the Community market. The directive distinguishes

between "new" and "existing" chemicals. Existing chemicals are those placed on the Community market before 18 September 1981 and are listed in the European Inventory of Existing Commercial Chemical Substances (EINECS). For new chemicals, the type and the amount of information to be provided depends on the level of production of the chemical (levels 0, 1 and 2); greater the production, more information is needed. For the first time in history, it includes toxicological and ecotoxicological data besides physico-chemical properties of the chemical. Ecotoxicological information required for the technical dossier of the "base set" (level 0) contains data about toxicity to fish, daphnids, and algae, as well as data about biotic and abiotic degradability of the substance (4). Additional information and tests such as prolonged

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toxicity study with daphnids and fish, and additional tests for accumulation and degradation are required for levels 1 and 2.

The aim of this study was to present a few examples of classification and labelling of selected chemicals, mainly based on experimental data. Inorganic chemicals (arsenic, copper) and organic chemicals (phenol, 1,4-butyne-1,4-diol) of different toxicity, biodegradability and bioaccumulation were selected for the evaluation of acute and chronic toxicity to algae, daphnids, and fish. The obtained toxicity data, ready biodegradability of phenol and 1,4-butyne-1,4-diol, and the octanol/water partition coefficient ($\log P_{ow}$) were used for their classification which was then compared with the existing classification (5) of the selected chemicals.

MATERIALS AND METHODS

The testing included arsenic (as As_2O_3), copper (as $CuCl_2 \cdot 2H_2O$), phenol, and 1,4-butyne-1,4-diol ($C_4H_6O_2$). Standard stock solutions were prepared by dissolving an appropriate amount of chemicals in distilled water (6). Five concentrations and a control were tested in each experiment. A preliminary test and two definitive trials were conducted for each species and chemical. In the definitive trials two replicates were used for each concentration and control.

Algal inhibition test

The green alga *Scenedesmus subspicatus* Chodat 1926 (CCAP 276/20) was obtained from the Culture Collection of Algae and Protozoa, Cumbria, United Kingdom. The stock culture of the alga was maintained in a nutrient solution according to Jaworski (cf. 7) at a constant room temperature of 21 ± 1 °C and under continuous fluorescent illumination (4000 lux) on an orbital shaker. In the toxicity test, algal growth was determined by measuring the cell density (8). Test flasks were constantly shaken and illuminated at 7000 lux. Algal density was determined by counting cells in a Bürker counting chamber after 0, 24, 48, and 72 hours. The inhibition of algal growth was determined by the comparison of areas under the growth curves, and the 72-hour IC_{50} was obtained by linear regression analysis.

Alga *Scenedesmus quadricauda* (Turpin) Brébisson was used for the toxicity testing of copper. The stock

culture of the alga was maintained in a nutrient solution according to Holm Hansen (cf. 9) under the same test conditions as *S. subspicatus*. After 72 hours of growth, the algal chlorophyll was extracted in hot ethanol and determined spectrophotometrically (10).

Acute and chronic toxicity tests with daphnids

Daphnia magna Straus 1820 were obtained from the Institut für Wasser, Boden und Lufthygiene des Umweltbundesamtes, Berlin, Germany. They were cultured at 21 ± 1 °C in 3-litre aquaria containing 2.5 L of modified M4 medium (11) and illuminated with fluorescent bulbs (approx. 1800 lux) 12 hours a day. They were fed with alga *Scenedesmus subspicatus* Chodat 1926 corresponding to 0.13 mg C/daphnia per day. Acute toxicity to daphnids (8) was evaluated by counting the immobile daphnids after a 48-hour exposure, and the EC_{50} value was calculated using the probit analysis (12).

Chronic toxicity to *Daphnia magna* was tested in a semi-static or a flow-through (13) exposure system according to the OECD Guideline (14). The room temperature was maintained at 21 ± 1 °C and the photoperiod was 16 hours of light and 8 hours of dark. On Mondays, Wednesdays, and Fridays the surviving daphnids were transferred to freshly prepared test solutions and fed with *Scenedesmus subspicatus* at a ratio of 0.15 mg C/day per daphnid. Young daphnids were counted daily and then removed. The endpoints of chronic toxicity test were the mortality of daphnids, appearance of the first offspring, and total offspring per female after 21 days. The daphnid reproduction data were analysed by the one-tailed Dunnett's test providing "no-observed-effect concentration" (NOEC) values (12).

Acute toxicity test with fish

Juvenile rainbow trout *Oncorhynchus mykiss* Walbaum 1990, about 6 cm in length, were obtained from the fish farm Povodje, near Ljubljana, Slovenia. Zebrafish *Brachydanio rerio* Hamilton Buchanan were obtained from a commercial supplier. They were acclimated to the test temperature at least seven days before the beginning of experiment. During acclimatisation the fish were fed with commercial fish food and the tanks were illuminated with fluorescent bulbs for 12 hours a day. The toxicity tests with the rainbow trout and zebrafish were conducted in a static exposure system at 12 ± 0.5 °C and 21 ± 1 °C, respectively (8). Zebrafish were exposed in three-litre aquariums

Table 1 An overview of criteria used for the classification of chemicals with regard to the aquatic environment

Fish 96-hour LC ₅₀ (mg/L) or daphnids 48-hour EC ₅₀ (mg/L) or algae 72-hour IC ₅₀ (mg/L)	Ready biodegradability (RB)	Bioaccumulation potential Log P _{ow} ≥3 or BCF ≥100	Classification R phrases Danger symbol (N)
≤1	NO	YES/NO	R50/53
	YES	YES	N
≤1	YES	NO	R50
			N
1-10	NO	YES/NO	R51/53
	YES	YES	N
10-100	NO	YES/NO	R52/53
	YES	NO	No classification*
Not available	NO	YES	R53
	YES	NO	No classification*

* A substance is not classified if it has either a proven potential to degrade rapidly in the aquatic ecosystem or an absence of chronic toxicity at the concentration of 1.0 mg/L (NOEC > 1 mg/L in a prolonged toxicity study with daphnid or fish).

containing 2.5 L of test solution and the rainbow trout in 40-litre tanks containing 35 L of sample. The end-point of the acute toxicity test was the survival of fish during 96 hours of exposure; dead fish were counted and removed every 24 hours. The LC₅₀ values were calculated using the probit analysis (12).

Classification of chemicals

The "base set" data of a dangerous substance are used for its classification in regard to possible adverse effects to aquatic environment. A substance can be classified as "harmful", "toxic" or "very toxic" to aquatic organisms depending on the 96-hour LC₅₀ for fish, 48-hour EC₅₀ for daphnids, and 72-hour IC₅₀ for algae (15). The classification is carried out according to the lowest effect concentration. Substances are classified as dangerous for the environment and labelled with the symbol N (dangerous for the environment) and an adequate risk phrase (R). If LC₅₀ or EC₅₀ or IC₅₀ are below 1 mg/L, a substance is classified as "very toxic to aquatic organisms" (danger symbol N, risk phrase R50). If the values obtained for toxicity are between 1 and 10 mg/L, a substance is considered "toxic to aquatic organisms" (danger symbol N, risk phrase R51). There where the LC₅₀ or EC₅₀ or IC₅₀ are between 10 and 100 mg/L, a substance is classified as "harmful to aquatic organisms" (risk phrase R52). Classification is also based on the assessment of ready biodegradability or bioaccumulation potential of a substance expressed as an octanol/water partition coefficient. A substance which is not readily biodegradable or its log P_{ow} is ≥3 or its bioconcentration

factor (BCF) is ≥100 "may cause long-term adverse effects in the aquatic environment" (risk phrase R53). A combination of risk phrases is also possible. For example, R50/53 means that the substance is very toxic to aquatic organisms and may cause long-term adverse effects in the aquatic environment. A scheme of criteria for the environmental classification of chemicals (1, 15, 16) is given in Table 1.

RESULTS AND DISCUSSION

Metals

The aquatic toxicity results and environmental fate data for arsenic and copper are given in Table 2. Arsenic caused adverse effects in all "base set" organisms. The highest toxicity was observed in daphnids. It is interesting to note that the difference between longer exposure (21 days) and acute exposure to arsenic was not significant in respect to daphnid survival and reproduction. Literature data about arsenic toxicity to algae point to an important influence of experimental conditions (e.g. test organism, chemical form, and test conditions). Concentrations between 0.16 and 1000 mg/L of arsenic (i.e. factor more than 6000) have been found to be of "no-effect" (17). The reported 48-hour EC₅₀ values for daphnids were in the range from 1.5 to 9.1 mg/L (18). Bartell and co-workers (19) obtained the LC₅₀ value at the concentration of 13.0 mg/L for the rainbow trout, which is similar to our result.

Based on our results (Table 2), arsenic could be classified as toxic to aquatic organisms with possible

Table 2 Aquatic toxicity, environmental fate data and classification of arsenic and copper

Arsenic	Copper
Aquatic toxicity – "Base set data" (21) Alga <i>Scenedesmus subspicatus</i> 72-hour IC ₅₀ = 34.7 mg/L Daphnids <i>Daphnia magna</i> 48-hour EC ₅₀ = 2.5 (2.4-2.7) mg/L Fish <i>Oncorhynchus mykiss</i> 96-hour LC ₅₀ = 15.3 (12.9-17.3) mg/L	Aquatic toxicity – "Base set data" Alga <i>Scenedesmus quadricauda</i> 72-hour IC ₅₀ = 0.18 mg/L Daphnids <i>Daphnia magna</i> 48-hour EC ₅₀ = 0.030 (0.022-0.038) mg/L Fish <i>Oncorhynchus mykiss</i> 96-hour LC ₅₀ = 0.48 (0.43-0.54) mg/L
Aquatic toxicity – Additional data Chronic toxicity <i>Daphnia magna</i> 21-day NOEC = 1.85 mg/L	Aquatic toxicity – Additional data Chronic toxicity <i>Daphnia magna</i> 21-day NOEC = 0.015 mg/L
Biodegradation Irrelevant	Biodegradation Irrelevant
R phrases, danger symbol Based on our results: R51/53, N Existing class. (5): R50/R53, N	R phrases, danger symbol Based on our results: R50/53, N Existing class. (5): R50/53, N

Concentrations given in this table refer to concentrations of arsenic and copper, and not to As₂O₃ and CuCl₂

long-term adverse effects in aquatic environment (R51/53). The existing, provisional classification of arsenic is R50/53 (5) due to high toxicity to algae in waters containing low nutrient levels (20). Further testing of chronic effects of arsenic may be necessary due to the NOEC value close to 1 mg/L.

Copper showed high toxicity to all tested organisms; the most sensitive were the daphnids with a 48-hour EC₅₀ at 0.030 mg/L. The strong toxic effect of copper was also confirmed by the 21-day NOEC of 0.015 mg/L (Table 2). Our high copper toxicity findings in tested organisms were comparable to literature which reports 4-day LC₅₀ values for juvenile rainbow trout ranging between 0.006 and 0.94 mg/L, depending on the water hardness, and 2-day LC₅₀ 0.058 mg/L for *Daphnia magna* (18). The toxicity of copper also largely depends on experimental conditions, and it seems that water hardness plays an important role (17,18). As the copper "base set data" for algae, daphnids, and fish were below 1 mg/L in our experiment, it may be classified as "very toxic to aquatic organisms, may cause long term adverse effects in the aquatic environment" (R50/53), which is in accordance with the existing classification (5).

General issues concerning the biodegradability and bioaccumulation potential expressed as log P_{OW} are not relevant for inorganic compounds while the bioconcentration factor (BCF) could be more appropriate for the bioaccumulation assessment.

Organic chemicals

Table 3 shows the aquatic toxicity, biodegradability and bioaccumulation data of phenol and 1,4 – butynediol. Phenol was toxic to the rainbow trout and daphnids, but not as much to algae. Literature shows (22) that fish (24-hour LC₅₀ 5.6-11.3 mg/L for rainbow trout as the most sensitive species) are more sensitive to phenol than daphnids (48-hour LC₅₀ 23 mg/L for *Daphnia magna*), which is similar to our results. According to the "base set data", phenol may be classified as harmful to aquatic organisms (R52). Chronic toxicity testing showed that the 21-day NOEC for daphnid reproduction in a flow-through system was 4.13 mg/L (13). Taking into account the additional data about chronic toxicity, phenol could not be classified considering the NOEC being more than 1 mg/L and a proven potential to degrade rapidly in the aquatic ecosystem (15). Environmental fate data for phenol suggest that it may not have long term effects in the aquatic environment due to ready biodegradability and log P_{OW} < 3 (Table 3). According to the existing classification, phenol is not classified as danger for the environment (5).

Our tests showed that 1,4-butynediol caused adverse effects to aquatic organisms at concentrations as high as tens of milligrams per litre. The inhibition of algal growth was not detected even at the highest tested concentration (Table 3). Literature reports little about the toxicity of 1,4-butynediol to aquatic

Table 3 Aquatic toxicity, environmental fate data and classification of phenol and 1,4-butyne diol

Phenol	1,4 – butyne diol
Aquatic toxicity – "Base set data" Alga <i>Scenedesmus subspicatus</i> 72-hour IC50=229 mg/L Daphnids <i>Daphnia magna</i> 48-hour EC50=27.9 (24.2 – 32.7) mg/L Fish <i>Oncorhynchus mykiss</i> 96-hour LC50=13.0 (11.9 – 14.1) mg/L	Aquatic toxicity – "Base set data" Alga <i>Scenedesmus subspicatus</i> 72-hour IC50 > 100 mg/L Daphnids <i>Daphnia magna</i> 48-hour EC50=30.2 (28.0 – 32.4) mg/L Fish <i>Brachydanio rerio</i> 96-hour LC50=76.1 (65.9 – 85.2) mg/L
Aquatic toxicity – Additional data Chronic toxicity <i>Daphnia magna</i> 21-day NOEC=4.13 mg/L	Aquatic toxicity – Additional data Chronic toxicity <i>Daphnia magna</i> 21-day NOEC=15.0 mg/L
Biodegradation readily biodegradable (23)	Biodegradation Not readily biodegradable (24)
Bioaccumulation Log P _{ow} =1.50 (22)	Bioaccumulation Log P _{ow} =-1.83 (22)
R phrases, danger symbol Based on our results: No classification Existing class. (5): No classification	R phrases, danger symbol Based on our results: R52/R53, N Existing class. (5): No classification

organisms (22). In regard to aquatic toxicity, it could be classified as a substance "harmful to aquatic organisms" (R52) which "may cause long-term adverse effects in the aquatic environment" (R53). Table 3 shows that 1,4-butyne diol is not readily biodegradable (24), yet it also has little bioaccumulation potential ($\log P_{ow} \leq 3$). The finding that the tested chemical is not readily biodegradable contradicts the data used for the existing classification according to which 1,4-butyne diol is not classified as dangerous for the environment due to ready biodegradation (5). Further study of the biodegradation potential of this substance is required.

CONCLUSIONS

Based on the obtained data, copper was classified as very toxic and arsenic as toxic to aquatic organisms, both with possible long term adverse effects in aquatic environment. 1,4-butyne diol could be classified as a substance harmful to aquatic organisms which may cause long-term adverse effects in the aquatic environment. Phenol was not classified due to its ready biodegradability and no bioaccumulation potential as well as due to low toxicity confirmed by additional chronic toxicity testing with daphnids. The

classification of copper and phenol that was based on the observed results is in accordance with the existing classification, whereas certain differences have been observed in the classification of arsenic and 1,4-butyne diol. The classification and labelling of chemicals in regard to aquatic environment is based on the proposed standard methods for measuring the toxicity of chemicals to aquatic organisms and their biodegradability. The "base set data" obtained by rather simple standardised test methods seems to be rather convenient for this purpose. Additional scientific data about environmentally relevant properties of arsenic and 1,4-butyne diol are required, which could lead to the revision of the existing classification of chemicals.

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Sažetak

TOKSIČNOST ODABRANIH KEMIKALIJA U VODENOJ SREDINI KAO TEMELJNI KRITERIJ ZA KLASIFIKACIJU NJIHOVE OPASNOSTI ZA OKOLIŠ

Zakonodavstvo Europske unije preporučilo je klasifikaciju opasnih kemikalija u svrhu zaštite zdravlja ljudi i okoliša. Opasne se kemikalije razvrstavaju na temelju fizikalno-kemijskih, toksikoloških i ekotoksikoloških svojstava. Autori su istraživali učinke različitih koncentracija arsena, bakra, fenola i 1,4-butindiola sa svrhom pokazivanja primjera kako se kemikalije mogu razvrstati na temelju rezultata pokusa na različitim algama, dafnijama i ribama. Pratili su akutnu i kroničnu toksičnost spomenutih kemikalija, njihovu biodegradabilnost i bioakumulaciju. Svoje su rezultate usporedili s postojećom klasifikacijom Europske unije. Utvrdili su da se bakar može klasificirati kao vrlo toksičan, a arsen kao toksičan za testirane vodene organizme te da oba mogu imati dugotrajne štetne učinke u vodenoj sredini. Fenol nije klasificiran zbog brze razgradljivosti, niskog bioakumulacijskog potencijala i niske toksičnosti. Klasifikacija bakra i fenola temeljena na dobivenim rezultatima u skladu je s postojećim normama Europske unije. Međutim, za arsen i 1,4-butindiol postoje razlike u rezultatima, pa će možda biti potrebna revizija postojeće klasifikacije zbog novih rezultata istraživanja okoliša.

KLJUČNE RIJEČI: *arsen, bakar, biološka razgradnja, 1,4-butindiol, fenol, označavanje opasnih tvari, vodeni okoliš*

REQUESTS FOR REPRINTS:

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