Bull. Environ. Contam. Toxicol. (1993) 50:878–882

Environmental Contamination and Toxicology

Toxicity Studies in Fertilized Zebrafish Eggs Treated with *N*-Methylamine, *N,N*-Dimethylamine, 2-Aminoethanol, Isopropylamine, Aniline, *N*-Methylaniline, *N,N*-Dimethylaniline, Quinone, Chloroacetaldehyde, or Cyclohexanol

G. Groth, K. Schreeb, V. Herdt, and K. J. Freundt*

Institute of Pharmacology and Toxicology, Faculty of Clinical Medicine, University of Heidelberg, Maybachstrasse 14–16, D-6800 Mannheim 1, Federal Republic of Germany

N-methylamine, N,N-dimethylamine, 2-aminoethanol, isopropylamine, aniline, N-methylaniline, N,N-dimethylaniline, quinone, chloroacetaldehyde, cyclohexanol are widely used in various industrial fields (Merck Index 1989). They may therefore contaminate the environment. Their possible embryotoxic and/or teratologic potency has not been published before (GRS 1991), except that of aniline (Price et al. 1985, Hardin et al. 1987). This gap could be filled by the investigation presented here. Fertilized zebrafish (Brachydanio rerio) eggs served as a well-recognized test object (Baumann and Sander 1984; Van Leeuwen et al. 1990) to elucidate morphological changes in the fish embryo during development and to discover reduction of the hatching rate induced by the above mentioned xenobiotics. From the results information important for the assessment of health risk are to be expected.

MATERIALS AND METHODS

The following chemicals (CAS numbers in parentheses) of reagent grade were used: N-methylamine (74-89-5), N,N-dimethylamine (124-40-3), 2-aminoethanol (141-43-5), isopropylamine (75-31-0), aniline-HCl (62-53-3), N-methylaniline (100-61-8), N,N-dimethylaniline (121-69-7), quinone (106-51-4), chloroacetaldehyde (107-20-0), cyclohexanol (108-93-0), $CaCl_2$ + 2 H_2O, KCl, $MgSO_4$ + 7 H_2O, $NaHCO_3$ from Merck, 6100 Darmstadt/FR Germany. The test substances were solved in reconstituted water.

Mature zebrafishes (Brachydanio rerio) commercially purchased (West Aquarium, 3422 Bad Lauterberg/FR Germany) were housed separated according to sex in aquaria (50 · 30 · 20 cm = 30 L) containing 2 mM $CaCl_2$ + 2 H_2O, 0.078 mM KCl, 0.5 mM $MgSO_4$ + 7 H_2O, 0.77 mM $NaHCO_3$ in demineralized water at 25° C. This reconstituted water with a pH of 8 ± 0.2 and a hardness of 200 mg/L as $CaCO_3$ was provided with bubbling air by a special pump. The diet was "Novo bel" and "Novo termia" (IBL, 6708 Neuhofen/FR Germany) for producing mature artemia larvas. Four female and eight male fishes were kept together overnight for spawing at dawn simulated by neon lighting. The eggs were fertilized extracorporally by the delivered sperm of the male fishes. The spawn was collected on the bottom of a glass box covered by a steel wire-net containing some small green glass trees. The eggs were separated and

*To whom correspondence should be addressed

cultured at 25° C individually using micro plates (Nunc, Roshilde/Denmark) containing 100 µL reconstituted water. At the 8-cell stage, test substances in 100 µL reconstituted water each were added after neutralization to pH 8 by HCl. Each test contained one control and up to seven concentrations of each chemical; 12 eggs were used per concentration or control. The eggs were observed microscopically for about 96 hr until hatching. Developmental stages were determined as described elsewhere (Hisaoka and Battle 1958). Marked signs of development, e.g. abnormalities, and deaths were recorded. The individual data from each test were plotted as means semi-logarithmically and the concentration without effect (no observed effect level, NOEL), the concentrations producing 50 % (LC_{50}) or 100 % (LC_{100}) embryo lethality were calculated using a special computer program based on a conventional method (Litchfield and Wilcoxon 1949). A t-test (P below 0.05 = significant), asymptotic normal distribution test, was used for statistical comparisons of differences.

RESULTS AND DISCUSSION

No deaths or morphological changes were observed in the controls (fish embryos without test substances). The concentrations without effects (NOEL), the LC_{50}, or the LC_{100} varied from agent to agent in some cases considerably (Table 1). The fish embryos were more sensitive to both methylanilines, quinone, and chloroacetaldehyde than to the other xenobiotics (Figure 1, Table 1). The respective death rates increased within a small concentration range showing steep slopes with the xenobiotics examined, except N-methylamine and N,N-dimethylamine (Figure 1). N-methylamine, N,N-dimethylamine, 2-aminoethanol, and isopropylamine induced no malformations in the embryos and the hatched fishes developed normally (Table 1). The other agents produced malformations in the fish embryos with lesions mainly of the heart or skeleton. Oedematous enlargement of the pericardial space with retardation of heart development and reduced blood flow was observed under the treatment with aniline, N-methylaniline, N,N-dimethylaniline, chloroacetaldehyde, and cyclohexanol (Table 1). A deformation of the skeleton and the muscle apparatus appeared in the embryos after administration of aniline, N-methylaniline, N,N-dimethylaniline, quinone, chloroacetaldehyde, or cyclohexanol (Table 1). A general retardation of the embryo development was seen under treatment with aniline, N-methylaniline, N,N-dimethylaniline, and cyclohexanol (Table 1). The underlying cause of death could not be determined explicitly in the embryos, even though an obvious association between malformations and death existed.

Conclusion: The embryotoxicity of aniline is enhanced considerably by alkylation of the chemical (aniline). NH_2-substitution of alkyl compounds leads to agents with less embryotoxicity.

Aniline-HCl caused slight fetotoxic effects: reduced body weight in mice (Hardin et al. 1987), increased liver weight and hematopoetic activity in rats (Price et al. 1985). But in both experimental studies no other malformations have been observed. These results in mammals (Hardin et al. 1987, Price et al. 1985) are in contrast to our morphological observations in zebrafish embryos.

Table 1. Results of the treatment at the 8-cell stage continously until hatching. The NOEL, LC_{50}, and LC_{100} were calculated using the values given in Figure 1.

Agent	Concentration Tested	NOEL	LC_{50} and 95 % Confidence intervals	LC_{100}	Malformation
N-Methylamine, mmol/L	2 - 500	3	22.9 (21.4 - 24.5)	100	no
N,N-Dimethylamine, mmol/L	1 - 200	1	8.8 (8.3 - 9.3) a)	100	no
2-Aminoethanol, mmol/L	10 - 800	20	60.3 (59.7 - 60.8)	100	no
Isopropylamine, mmol/L	0.1 - 200	10	91.2 (90 - 92.4)	200	no
Aniline, mmol/L	0.2 - 20	1	9.3 (9.2 - 9.5) a)	17	b), c), d)
N-Methylaniline, µmol/L	0.1 - 5	0.2	0.71 (0.69 - 0.72)	1	b), c), d)
N,N-Dimethylaniline, µmol/L	0.08 - 4	0.33	1.51 (1.49 - 1.54)	2	b), c), d)
Quinone, µmol/L	0.5 - 10	0.8	4.37 (4.30 - 4.43)	5	c)
Chloroacetaldehyde, µmol/L	1 - 100	6	42.7 (41.8 - 43.6)	60	b), c)
Cyclohexanol, mmol/L	1 - 30	3	13.8 (13.7 - 13.9)	16	b), c), d)

a) not statistically different from one another, t-test (P below 0.05 = significant)
b) oedematous enlargement of the pericardial space in about 20 % of all treated embryos
c) deformation of the skeleton and muscle apparatus in approximately one third of all treated embryos
d) retardation of body development in about 20 % of all treated embryos

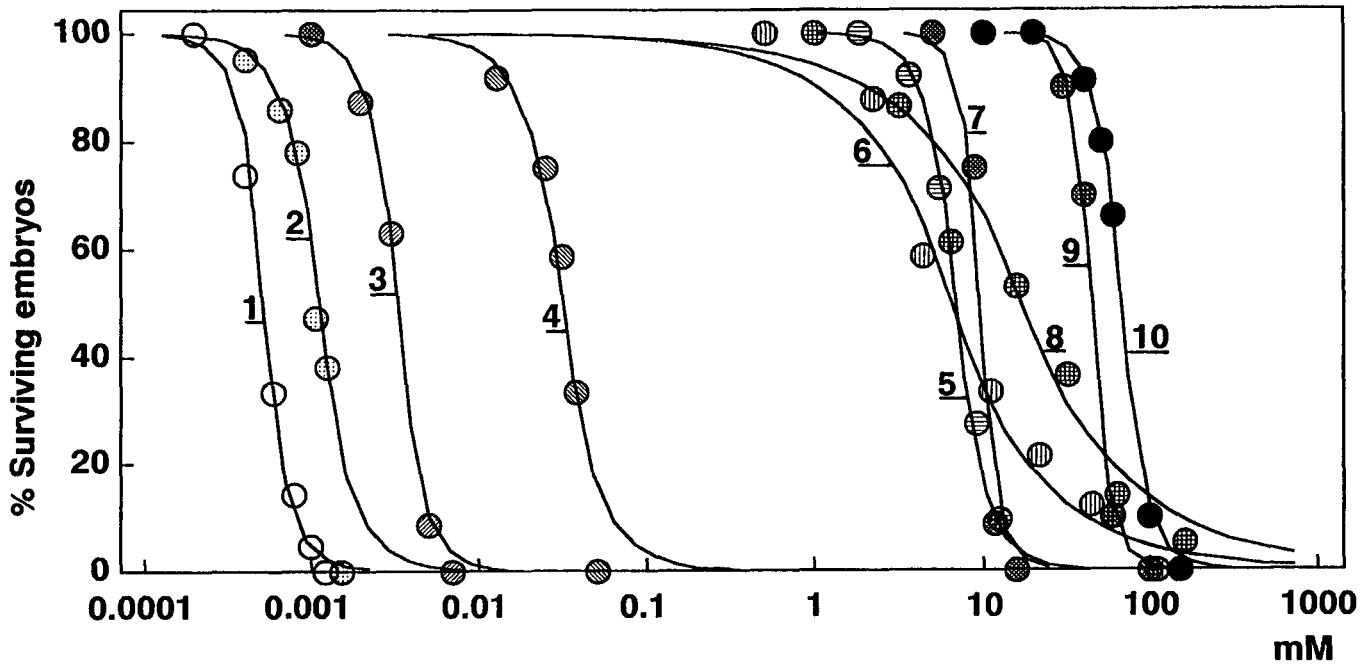

Figure 1. Semi-logarythmic plot of surviving fish embryos as percent against the concentrations of the agents in the fish buffer. Each point represents the mean (SEM was below 5 % and is not shown).
1: N-methylaniline, 2: N,N-dimethylaniline, 3: quinone, 4: chloroacetaldehyde, 5: aniline-HCl,
6: N,N-dimethylamine, 7: cyclohexanol, 8: N-methylamine, 9: 2-aminoethanol, 10: isopropylamine

The results of the present study in zebrafish embryos are to be considered more under ecotoxicological aspects, since a close correlation has been reported between tropical zebra fish (Brachidanio rerio) and cold water rainbow trout (Salmo gairdneri) embryotoxicity for a limited number of chemicals (Van Leeuwen et al. 1990). Since early developmental stages in the life cycles of fish may be very sensitive to the action of xenobiotics (Van Leeuwen et al. 1990), embryotoxic observations in experimental fishes may speed up risk assessment processes. In this context, results obtained in fish embryos may have a predictive value for the assessment of possible hazards of chemicals in wildlife and humans.

REFERENCES

Baumann M, Sander K (1984) Bipartite axiation follows incomplete epiboly in zebrafish embryos treated with chemical teratogens. J Exp Zool 230: 363 - 374

GRS German Research Society (1991) Maximum concentrations at the workplace and biological tolerance values for working materials. Report No. XXVII. Commission for the investigation of health hazards of chemical compounds in the work area. VCH Verlagsgesellschaft, Weinheim / FR Germany

Hardin BD, Schuler RL, Burg JR, Booth GM, Hazelden KP, MacKenzie KM, Piccirillo VJ, Smith KN (1987) Evaluation of 60 chemicals in a preliminary developmental toxicity test. Teratog Carcinog Mutag 7:29 - 48

Hisaoka KK, Battle HI (1958) The normal developmental stages of the zebrafish, Brachydanio rerio (Hamilton-Buchanan). J Morph 102:311 - 328

Litchfield JT, Wilcoxon F (1949) A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 96:99 - 113

Price CJ, Tyl RW, Marks TA, Paschke LL, Ledoux TA, Reel JR (1985) Teratologic and postnatal evaluation of aniline hydrochloride in the Fischer 344 rat. Toxicol Appl Pharmacol 77:465 - 478

The Merck Index (1989) An encyclopedia of chemicals, drugs, and biologicals, 11 ed. Merck & Co Inc, Rahway, New Jersey USA

Van Leeuwen CJ, Grootelaar EMM, Niebeek G (1990) Fish embryos as teratogenicity screens: a comparison of embryotoxicity between fish and birds. Ecotoxicol Environ Safety 20:42 - 52

Received June 23, 1992; accepted December 14, 1992.