Toxicity of aliphatic amines on the embryos of zebrafish *Danio rerio* - experimental studies and QSAR

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1 INTRODUCTION

Amines and their derivatives are compounds which are extensively used as drugs, cosmetics, dyes, in synthesis of pesticides and as synthetic intermediates. Aliphatic amines are considered to be strong organic bases (Nelson, 1985).

In 1992 the production of primary fatty amines with a chain length of C_8 to C_{18} was about 8,000 tonnes and, for example the production of octylamine, was under 500 tonnes (BUA, 1994). About 75 % of the primary fatty amines are used as intermediates for synthesising ethoxylated fatty amines. These are widely used as cationic, surface-active substances, for example in auxiliary agents for dyeing and textile, as additives for mineral oil and as antistatic agents for plastics. The remaining 25 % were directly used in form of their salts, mainly as flotation agents, as dispersing agents for pigments or as corrosion inhibitors (BUA, 1994).

In the United States of America two to three thousand tonnes of cyclohexylamine are used each year as corrosion inhibitor in steam lines and boiler heating systems. The substance can reach into the air via steam humidification of indoor air (Orlando and Lao, 1993). Cyclohexylamine is suspected to have a teratogenic, mutagenic and carcinogenic potential (Klaasen *et al.*, 1986). A mammalian fertility study suggests that cyclohexylamine targets Sertoli cells in the testes (Creasy *et al.*, 1990).

The primary fatty amines may enter the geosphere due to their use as flotation agents and reach the soil directly. Total emissions of fatty amines into the hydrosphere from the manufacturing and from chemical processing (excluding salt formation) amounted to less than 200 kg per year, or, in respect to the individual fatty amines, less than 30 kg for each (BUA, 1994). Primary fatty amines are interface-active compounds due to their ability to form protons simply and this property explains the strong adsorption potential. Thus, a high geoaccumulation potential due to their physi- and chemisorption on inorganic soil components can be expected (BUA, 1994).

Between 1994 and 1996 the primary aliphatic methylamine was measured with concentrations of 0.15 μgL^{-1} to 0.29 μgL^{-1} in the river Elbe (Pietsch, 1997).

The production of morpholine, a secondary aliphatic amine included in this study, was about 12,000 tonnes in 1988 in Germany and more than 75 % is exported (BUA, 1990). This compound is mainly used as corrosion inhibitor or solvent. The amounts entering the atmosphere

during manufacture are likely to be small. However, the entry of morpholine into the environment is accompanied by an non-quantifiable amount of the nitrosation product, N-nitrosomorpholine (BUA, 1990). Nitrosation products are known to have a carcinogenic potential. No data about bio- and geoaccumulation of morpholine were available.

Piperidine, also a secondary aliphatic amine, is a member of a group of biogenic amines and is present in the central nervous system of both invertebrate and vertebrate (Giacobini, 1976). It is a volatile base with nicotine-like action and was regarded as endogenous "synaptotropic substance" (von Euler, 1945; as cited in Giacobini, 1976).

In the rivers Main and Rhine maximum concentrations of 4 μgL^{-1} diethylamine and 4 μgL^{-1} diisopropylamine, two further secondary amines, were measured, respectively (Scholz, 1992).

Tributylamine, a tertiary aliphatic amine, is used as intermediate, as a catalyst and acid acceptor in organic synthesis and polymerisation, as corrosion inhibitor and as solvent and auxiliary for isolating and purifying antibiotics (BUA, 1988). Discharge into the environment is estimated to be less than 5 kg per year in Germany and occurs almost via waste water. No information about the occurrence in the atmosphere or in soil and sediments were available. In the 1970s tributylamine was found in the river Rhine in a concentration range of 0.1 to 1 μgL^{-1} (BUA, 1988).

Dimethylethylamine, also a tertiary aliphatic amine, is used as a catalyst in the polymerisation of polyurethane (Lundh *et al*., 1997). It is a highly water-soluble and volatile compound and its concentration in the ambient air may be high (Lundh, *et al*., 1991).

Most toxicity tests with aliphatic amines were performed using various fish species including salmonid fish such as the rainbow trout *Oncorhynchus mykiss* and cypriniformes species such as the Medaka *Oryzias latipes*, the fathead minnow *Pimephales promelas* and the creek chub *Semotilus atromaculatus*, respectively (Gillette *et al.*, 1952; Calamari *et al.* 1982; Tonogai *et al.*, 1982; Wellens, 1982; Brooke *et al.*, 1984; Canton *et al.*, 1984; Geiger *et al.*, 1986; Geiger *et al.* 1988; Geiger *et al.*, 1990; Van Leeuwen *et al.* 1990; Groth *et al.* 1993; Broderius *et al.*, 1995). In general, the available toxicity data for a single fish species cover only a minor part of aliphatic amines. Besides, a comparison of these data is difficult because of the different test designs and the different fish species used. Therefore, to establish a greater and valid data base for QSAR the toxicity of aliphatic amines was determined using the embryotest (*Dar*T – *Danio rerio* Toxicity Assay) with the zebrafish *Danio rerio*. The zebrafish has been used as a model in numerous studies in the fields of molecular genetics, vertebrate biology as

well as in developmental, neurobiology and transgenic research (Roosen-Runge, 1938; Hisaoka and Battle, 1958; Laale, *et al.*, 1977; Sander, 1983; Nagel, 1988; Kimmel *et al.*, 1995; Westerfield, 1995; Lele and Krone, 1996; Goolish *et al.* 1999; Wixon, 2000).

Testing with embryos of the zebrafish *D. rerio* enables to define lethal as well as sublethal effects of chemicals on fish. The *Dar*T was developed as an alternative method to replace the acute toxicity tests of juvenile and adult fish for ethical reasons (Schulte and Nagel, 1994; Nagel and Isberner, 1998). Within 48 hours lethal effects of single substances (Görge and Nagel, 1990; Groth *et al.*, 1993; Schulte and Nagel, 1994; Wiegand *et al.*, 2000) or mixtures of substances (Ensenbach and Nagel, 1995) can be determined. Furthermore, specific modes of action of chemicals can be determined with a differentiated examination of sublethal effects like malformations at different stages of the ontogenetic development (Cheng *et al.*, 2000; Samson *et al.*, 2001). The principle of the test includes the individual exposure of the embryos to a range of concentrations of a test substance dissolved in water or in diluted waste water. The test design enables the determination of LC_{50} (median lethal concentration) and EC_{50} (median effect concentration). In addition, the embryos of the zebrafish are included in studies for teratogenicity screens (Van Leeuwen *et al.*, 1990; Herrmann, 1993; Bachmann *et al.* 2001), for the assessment of waste water (Friccius *et al.*, 1995), and as model to assess the toxicity of sediments to vertebrates (Ensenbach, 1998).

The relationship between the biological activity of molecules (e.g. 96-h LC_{50}) to their chemical structures and corresponding chemical and physicochemical properties can be described by mathematical models in quantitative structure-activity relationships (QSARs) (Lipnick, 1995). These relationships are becoming of increasing interest for environmental hazard assessment (Karcher and Devillers, 1990; Hermens and Opperhuizen, 1991; Jäckel and Klein, 1991; Verhaar *et al.*, 1992; Lipnick, 1995). The ultimate rationale is the establishment of causal relationships between features of the chemical structures and the observed effects or activities (Nendza, 1998). These relationships can be used to predict the behaviour, accumulation potentials, and toxicity of chemicals so far untested, and further to rationalise future experiments (Könemann, 1981; Saarikoski and Viluksela, 1981; Nendza, 1991). The effects of toxicants on biota depends on their hydrophobic, polar and electrostatic character and can be determined by reactive transient interactions, hydrogen bonding, covalent binding and/or steric fit to the interaction site (Nendza and Russom, 1991).

The experimental approach is the best way to determine the mode of action and can conclude the observation of symptoms elicited by an organism during a toxicity test (Boxall *et al.*, 1997). McKim and coworkers (1987) developed the fish acute toxicity syndromes (FATS) method based on acute tests with fish (*P. promelas*) in which a compound is assigned to one of seven modes of action: narcotics, polar narcotics, uncouplers of oxidative phosphorylation, respiratory membrane irritants, acetyl cholinesterase inhibitors, toxicants that affect central nervous system and respiratory blockers.

A broad classification scheme was developed by Verhaar and coworkers in 1992 in which compounds are assigned to one of four general classes of toxic mode of action corresponding to short-term exposures with lethality as endpoint: inert chemicals, less inert chemicals, reactive chemicals and specific-acting chemicals. Secondary and tertiary aliphatic amines were classified as inert compounds (narcotics or baseline toxicity), because of their non-specific mode of action and a general coherency between lipophilicity and toxicity was suggested. Primary aliphatic amines are slightly more toxic than baseline toxicity and were classified as less inert compounds acting by a "polar narcosis" mechanism. In general, narcosis in aquatic organisms is described as a non-specific reversible functional disturbance of bilogical membranes due to the accumulation of chemicals in hydrophobic phases within the organism (van Wezel and Opperhuizen, 1995). The membrane lipids and protein components have to be regarded as the most relevant targets for environmental toxicants with a narcotic mode of action (Nendza, 1998), and narcosis can be caused by chemicals from different classes. QSARs for non-reactive non-polar or polar chemicals are well established (Könemann, 1981; Veith *et al.*, 1983; Hermens *et al.*, 1984a; Veith and Broderius, 1987 and 1990; Bradbury *et al.*, 1989; Schultz *et al.*, 1989; Nendza and Russom, 1991; van der Zandt *et al.* , 1994; Verhaar *et al.*, 1996; Ramos *et al.*, 1997; Zhao *et al.*, 1998; Freidig and Hermens, 2000).

For example, QSAR studies were performed for selected aliphatic and aromatic amines. Schultz and coworkers (1991b) investigated the relative toxicity of 12 aliphatic and 12 aromatic amines in the 48 h *Tetrahymena pyriformis* static population growth impairment assay. They found a good relationship between the toxicity and the lipophilicity. Newsome and coworkers (1991) found that the 96-h LC_{50} of amines for the fathead minnow *Pimephales promelas* could be best described by using the log K_{ow} or the valence first-order connectivity index ($^1\chi^v$) as descriptors. The toxicity using 48-h EC_{50} values for several species of *Daphnia* could be also best described by using the lipophilicity (Newsome *et al.*, 1993). Within the context of mammalian toxicity Jäckel and Klein (1991) developed a QSAR using oral toxicity data of

aliphatic amines for rats. In their study the toxicity could be best described by a bilinear log K_{ow}-dependent relationship. Greim and coworkers (1998) compiled several toxicological properties for 37 structurally related aliphatic amines on the basis of the acute oral toxicity for rats.

Nevertheless, only a few data are available for the group of aliphatic amines. Especially in the aquatic field the data for fish are very heterogeneous due to the variety of species, test durations and designs used.

The aim of this study was to investigate the effects of selected 13 primary, 13 secondary and 10 tertiary aliphatic amines on the embryonic development of the zebrafish within 48 h and to record possible specific toxicity patterns caused by the substances. The major advantage of this dataset over other toxicity data compilations is that variation in measured bioactivity (LC_{50}) is due to differences in toxic potency and not due to different test protocols. Further, a suitable quantitative model to describe the relationship between chemical structure and toxicity to the zebrafish embryos for the aliphatic amines should be developed.

2 MATERIALS AND METHODS

2.1 The zebrafish *Danio rerio*

The zebrafish *Danio rerio* is a small cyprinid found in tributaries and branches of the Ganges River in South-East Asia (Eaton and Farley, 1974). Adults measure 3-5 cm in length and thrive in both soft and hard water. At 26°C the zebrafish grows quickly and reaches maturity within three months. This species is easily obtainable, inexpensive, readily maintainable and, under appropriate conditions, will provide a large number of non-adherent and transparent eggs (Laale, 1977). One female lays approximately 50 - 200 eggs per day. The zebrafish is a r-strategist (Nagel, 1993).

The embryonic development was described in numerous studies (Roosen-Runge, 1938; Hisaoka and Battle, 1958; Laale, 1977; Thomas and Waterman, 1978; Kimmel *et al.*, 1988; Kimmel *et al*., 1995) and is the basis for the interpretation of effects caused by environmental pollutants.

The *Danio rerio* egg is telolecithal, and cleavage is meroblastic and discoidal. Shortly after fertilization, cytoplasm of the egg accumulates at the animal pole where it surrounds the nucleus of the zygote. Only this portion of egg cytoplasm, the so called blastodisc undergoes cleavage, whereas the yolk rich zone is excluded from cleavages. In Table 1 the stages of embryonic development of zebrafish embryos are summarised.

Table 1: Stages of embryonic development of the zebrafish *Danio rerio* at 26±1°C.

Time [h]	**Stage**	**Characterisation** (after Kimmel *et al.*, 1995)
0	Fertilisation	zygote
0	Zygote period	cytoplasm accumulates at the animal pole, one-cell-stage
¾	Cleavage period	discoidal partial cleavage;
		1. median vertical division: two-cell-stage
1		2. vertical division: four-cell-stage
1¼		3. vertical and parallel to the plane of the first: eight-cell-stage
1½		4. vertical and parallel to the second plane of division: 16-cell-stage
2	Blastula period	start of blastula stage
3		late cleavage; blastodisc contains approximately 256 blastomeres
4		flat interface between blastoderm and yolk
5¼	Gastrula period	50 % of epibolic movements; blastoderm thins and interface between periblast and blastoderm becomes curved
8		75 % of epibolic movement
10		epibolic movement ends, blastopore is nearly closed
10½	Segmentation period	first somite furrow
12		somites are developed, undifferentiated mesodermal component of the early trunk, tail segment or metamere
20		muscular twitches; sacculus; tail well extended
22		site to side flexures; otoliths
24	Pharyngula period	phylotypic stage, spontaneous movement, tail is detached from the yolk; early pigmentation
30		reduced spontaneous movements; retina pigmented, cellular degeneration of the tail end; circulation in aortic arch 1
36		tail pigmentation; strong circulation; single aortic arch pair; early motility; heart beating starts
72-96	Hatching period	heart beat regularly; yolk extension beginning to taper; dorsal and ventral stripes meet at tail; segmental blood vessels; thickend sacculus walls with two chambers; foregut developments

In the following typical stages of the embryonic development of the zebrafish are shown (Figure 1 – 4).

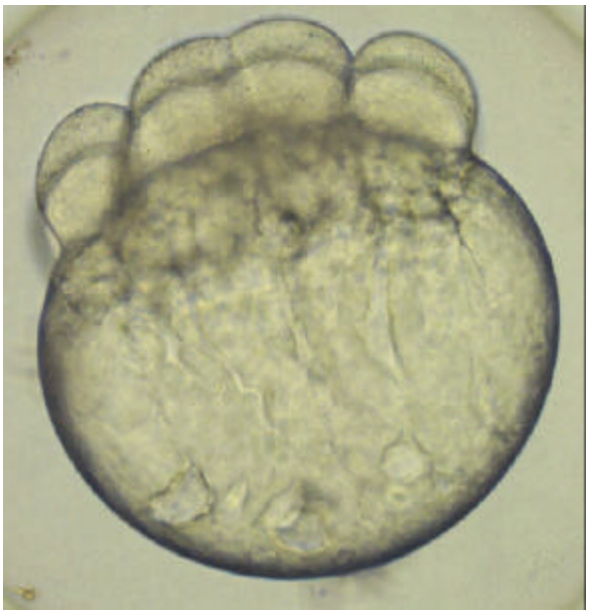


Figure 1: Eight-cell-stage of an embryo of *Danio rerio* approximately 1¼ h after fertilisation (from Zeller, 1995).

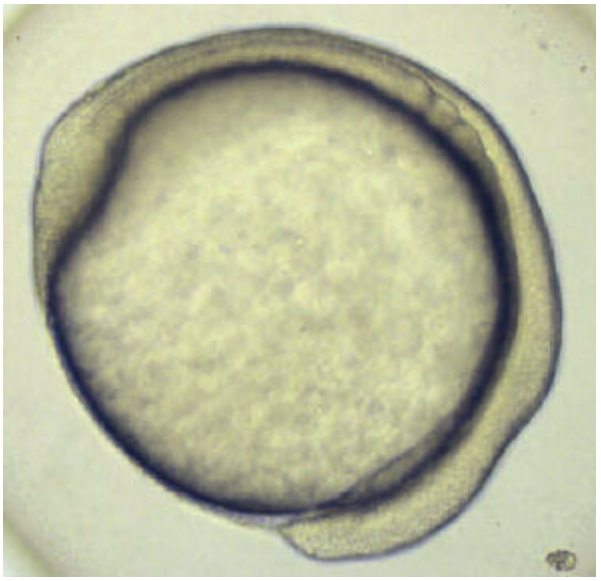


Figure 2: Segmentation phase of an embryo of *Danio rerio* approximately 12 h after fertilisation. To be seen are the head- and tail region as well as the somites (from Zeller, 1995).

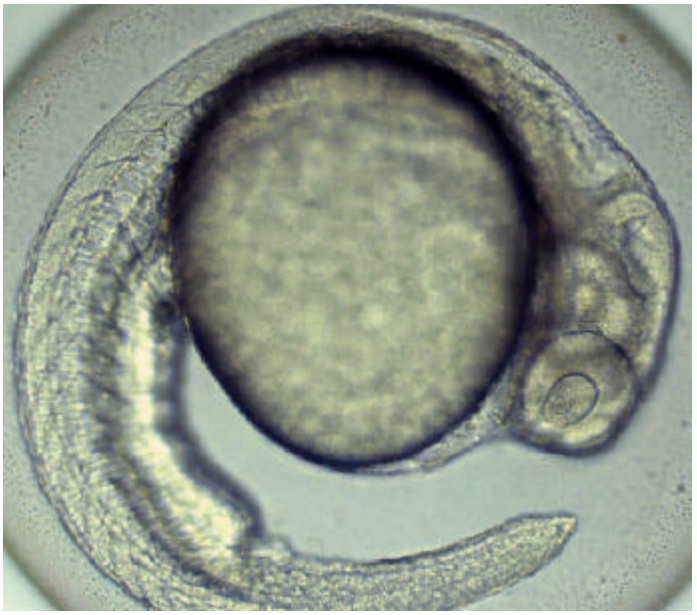


Figure 3: Normal developed embryo of *Danio rerio* after 24 h. The tail is detached from the yolk and spontaneous movement starts at this time (from Zeller, 1995).

Figure 4: Normal developed embryo of *Danio rerio* after 48 h. Pigmentation of the eyes and skin due to melanophores, the sacculus (SC) containing two otoliths as well as the completely developed and well structured spine are to be seen. At this stage blood circulation and regular heart beats can be observed.

2.1.1 Culture conditions

A breeding stock of non-treated, mature zebrafish was used for egg production. Females and males (total N = 30) were kept at a ratio of 1:2 in a 70 L glass aquarium filled with charcoal filtered tap water with an oxygen saturation of more than 80 %. The culture conditions were 26 ± 1°C at a 12 hour day/night light regime. Optimal filtering rates were adjusted using a filter system (Eheim, Deizisau, Germany). The fish were fed with dry flakes (TetraMin®, Tetra Werke, Melle, Germany) twice per day, and *ad libitum* with nauplia larvae of *Artemia salina* once a day (Sanders®, Sanders Brine Shrimp Company, Utah, USA). To ensure optimal water quality remaining food was removed and 10 L of the water were replaced by aerated tap water daily.

2.1.2 Egg production and differentiation

To prevent the eggs from being cannibalised by the adult zebrafish the spawn traps were covered with a stainless steel mesh (3 mm, diam.). Plant imitations made of green glass were used as spawning substrate. The spawning and fertilisation took place within 30 minutes after light was turned on in the morning. 30 – 60 minutes after spawning the egg traps were removed and the eggs were collected in a plastic mesh sieve. A single mature female lays 50 – 200 eggs per day. At the culture conditions described above fertilised eggs undergo the first cleavage after approximately 15 min and consecutive synchronous cleavages form 4, 8, 16, and 32 cell blastomeres. At this stages fertilised eggs can be identified clearly and only these were used for the experiments.

2.2 *Dar*T – The *Danio rerio* toxicity assay

The embryotest procedure described by Schulte and Nagel (1994) was applied. Following initial range-finding experiments, the toxicity of a chemical substance can be determined by using 24-well multiplates (NUNC, Wiesbaden, Germany). After preparing a stock solution of the test substances five concentrations were tested using a constant factor at least 1.2, on one multiplate each.

40 eggs were transferred to the test solutions about 60 minutes after light was turned on. Fertilised eggs were separated from the non-fertilised and placed in the multiplate wells with a pipette using a stereo microscope (magnification 4 – 40x, SZ 40 45 TR, Illumination Base SZ 17 ILLK, Olympus Optical Co., Ltd., Tokyo, Japan). 20 fertilised eggs were placed individually in 2 mL of the respective test solutions to exclude mutual influences. The remaining four wells of each plate were used as internal control filled with dilution water amounting to a total of 20 controls per test. The dilution water corresponded to the reconstituted water according to ISO – standard 7346/3, which was diluted 1:5 using deionised water (Nanopure, Millipore, Milford, MA, USA). After this procedure the multiplates were covered with a self-adhesive foil (NUNC, Wiesbaden, Germany) and incubated at 26°C ± 1°C (Heraeus, BK 6160, Hanau, Germany). Both lethal and sublethal endpoints were recorded using a dissecting microscope (magnification 40 – 150x, Olympus, IMT 2, Tokyo, Japan) within 48 h of the embryotest (Table 2). The test is classified as valid, if 90 % of the embryos in the control treatments showed neither sublethal nor lethal effects. Only for the test with tributylamine ethanol was used as solvent and an additional solvent control was performed.

Under the assumption that the non-ionised species of a compound can diffuse better through membranes the tests with the amines were carried out without adjusting the pH to 7.5. Prior to the test pH and oxygen concentration were measured in the treatments and the control media (O_2-sensor TriOximatic, pH-electrode E96, WTW, Weilheim, Germany).

Table 2: Lethal and sublethal endpoints for evaluating the toxicity of aliphatic amines on the embryo of *Danio rerio* within 48h (according to Schulte and Nagel, 1994).

Toxicological endpoints	**Exposure time (h)**		
	8	**24**	**48**
lethal			
coagulation	•	•	•
tail not detached		•	•
no somites		•	•
no heart beat			•
sublethal			
completion of gastrula	•		
development of eyes		•	•
spontaneous movement		•	•
sacculus with otoliths		•	•
deformities		•	•
blood circulation			•
pigmentation			•
oedema			•

2.3 Test Substances

Just as all other amines aliphatic amines are derivatives of ammonia. The functional group within the primary amines is the amino-group. The secondary amines are distinguished by the secondary amino-group, which means that one hydrogen was replaced by another substituent. In the case of tertiary amines a tertiary nitrogen atom is bond to three substituents (Figure 5).

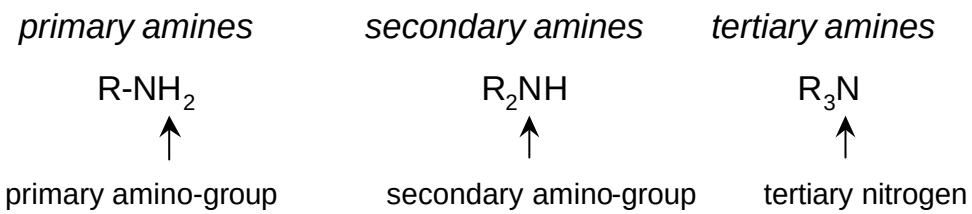


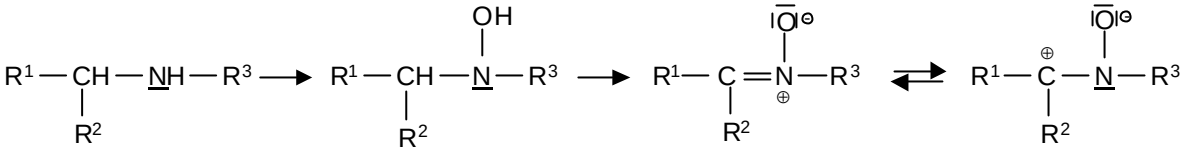
Figure 5: Classification scheme of the aliphatic amines into primary, secondary and tertiary amines, and their functional groups.

The biotransformation of amines is catalysed by the cytochrom P 450-system which is located in the endoplasmatic reticulum, in the membranes of mitochondria and in plasmatic membranes of prokaryotes . The cytochrom P 450 is an important system for the metabolism of foreign compounds. Within this system the mixed-function-oxidase (MFO) catalyses CH-hydroxylations including N- and O-dealkylations, π-bond oxygenations such as aromatic hydroxylations, epoxidations, and thiophosphate oxidations, and also thioether and nitrogen oxidations (Brattsten, 1979). Gorrod (1973) reported that all amines with pK_a > 8 will be metabolised by the amine oxidase.

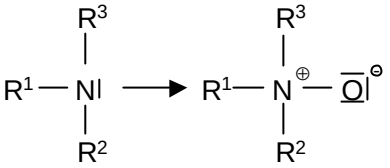
The N-oxidation (Bonse and Metzler, 1978) is the most important metabolic way for N-containing chemicals in humans and animals. Primary amines can be oxidised to hydroxylamines and then to oximes:

$$R\text{-}CH_2\text{-}NH_2 \rightarrow R\text{-}CH_2\text{-}NH\text{-}OH \rightarrow R\text{-}CH{=}N\text{-}OH$$

Secondary amines can be oxidised to hydroxylamines and then, if an hydrogen atom is available at the α-C atom, to nitrones:



Tertiary amines can be transformed by microsomal enzymes to *N*-oxide-metabolites which then can be further desalkylated and/or reduced:



The linear primary amines C_2 to C_{18} are readily biodegradable. The degradability of secondary and tertiary amines depends on the length and type of the alkyl group. Higher secondary amines are less degradable than primary amines (Zahn and Wellens, 1980). Yoshimora and coworkers (1980) found that tertiary amines C_4 to C_{18} are particularly unbiodegradable although this fact could only be shown for triethylamine (Chudoba *et al.*, 1969).

The most important property of aliphatic amines is their basic character. If an aliphatic amine is dissolved in water the pH will increase due to the protonation and alkylammonium ions and hydroxide ions will be formed. Using the Brønsted-Lowry concept the alkylammonium ion acts as an acid which donates a proton to the hydroxide ion, whereas the hydroxide ion acts as a base. Both are related by the gain and the loss of a proton, and are therefore a conjugate acid-base pair (Figure 6):

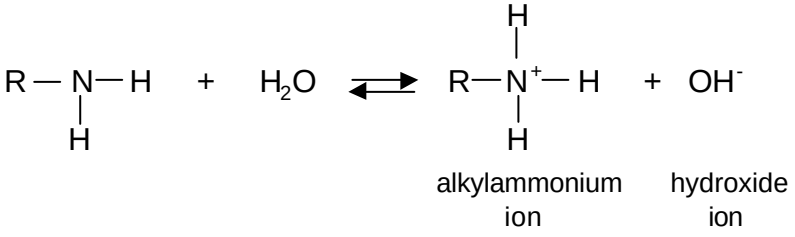


Figure 6: Theoretical scheme of the dissociation of amines in water.

In theory, it can be expected that the basicity decreases from the primary to the tertiary amines: $R\text{-}NH_2 > R_2NH > R_3N$.

On the other hand the steric effects of solvation intensifies the basicity from the tertiary to the primary amines:

$$R_3N > R_2NH < R\text{-}NH_2.$$

Therefore, the secondary amines are slightly more basic then the primary and the tertiary amines as can be seen in the pK_a-values (Table 3).

For organic acids and bases, the acidity constant provides an indication of the amount of ionised and unionised species of the substance that will be available at a given pH. As a rule, if the pH is equal to the pK_a aliphatic amines will be 50 % ionised and 50 % unionised. Because of the influence of pK_a on both transport through biological membranes and toxicity the measured pH values at the beginning of the exposure can be used to calculate the degree of ionisation of the aliphatic amines using the Henderson-Hasselbalch equation 1:

(1) $$\% \text{ ionisation} = \frac{100}{1+10^{(pH-pK_a)}}$$

The basic character of the amines is important for the performance of experiments in aqueous phases. In consequence, prior to testing the effects of aliphatic amines the sensitivity of the embryos of zebrafish to basicity should be determined. The effects of basicity were investigated using a 0.1 n NaOH solution. The reconstituted water (see chapter 2.2) was adjusted to the following pH´s: 8.0; 8.5; 9.0; 9.5; 10.0; 10.5; 11.0; 11.5 and 12.0. After 48 h lethal and sublethal effects were recorded (see chapter 2.2, Table 2).

Thirtysix branched and unbranched saturated aliphatic amines were used for embryo toxicity testing. The substances were purchased from Merck (Darmstadt, Germany), Acros (Brussels, Belgium) or Sigma-Aldrich® GmbH (Deisenhofen, Germany). The purity was ≥ 95 %.

In Table 3 the CAS-No., chemical formula, structural formula, the simplified molecular input entry system-code (SMILES), the pK_a-values, the molecular weight, and the log K_{ow} of the amines are presented. The log K_{ow} as a measure of the lipophilicity of the amines ranged from – 0.56 to 4.46 and is given as estimated value using the program KowWin (Version 1.90) and, if possible, as experimental value obtained from several sources.

Table 3: CAS-No., chemical formula, structural formula, simplified molecular input line entry system-code (SMILES), molecular weight (MW) given in $gmol^{-1}$, pK_a-values, and the log K_{ow} with estimated (est.) and, if available, experimental (exp.) values for aliphatic amines.

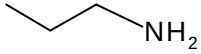
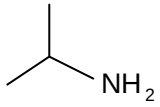
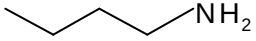
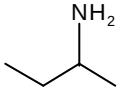
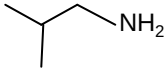
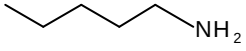
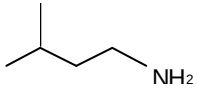
Substance/CAS-No.	**Formula**	**Structures**	**SMILES**	**pK_a**	**MW**	**log K_{ow} est.[f] / exp.**
Primary amines						
n-Propylamine [107-10-8]	C_3H_9N		NCCC	10.7[a]	59.11	0.34 / 0.48[g]
Isopropylamine **[75-31-0]**	C_3H_9N		NC(C)C	10.6[b]	59.11	0.27 / 0.26[h]
n-Butylamine [109-73-9]	$C_4H_{11}N$		NCCCC	10.8[a]	73.14	0.83 / 0.97[g]
sec-Butylamine [13952-84-6]	$C_4H_{11}N$		NC(CC)C	10.7[c]	73.14	0.76 / 0.74[i]
Isobutylamine [78-81-9]	$C_4H_{11}N$		NCC(C)C	10.6[a]	73.14	0.76 / 0.73[h]
n-Pentylamine [110-58-7]	$C_5H_{13}N$		NCCCCC	10.6[a]	87.17	1.33 / 1.49[g]
Isopentylamine [107-85-7]	$C_5H_{13}N$		NCCC(C)C	10.6[d]	87.17	1.25 /

Table 3: (Continued)

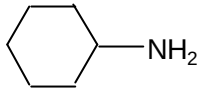
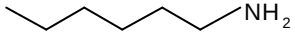
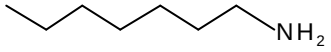
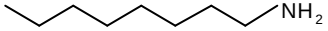
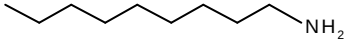
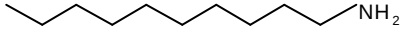
Substance/CAS-No.	**Formula**	**Structures**	**SMILES**	**pK_a**	**MW**	**log K_{ow} est.[f] / exp.**
Primary amines						
Cyclohexylamine [108-91-8]	$C_6H_{13}N$		NC(CCCC1)C1	10.6[c]	99.18	1.63 / 1.49[h]
n-Hexylamine [111-26-2]	$C_6H_{15}N$		NCCCCCC	10.6[a]	101.19	1.82 / 2.06[g]
n-Heptylamine [111-68-2]	$C_7H_{17}N$		NCCCCCCC	10.7[a]	115.22	2.31 / 2.57[g]
n-Octylamine [111-86-4]	$C_8H_{19}N$		NCCCCCCCC	10.7[a]	129.25	2.8 / 2.9[g]
n-Nonylamine [112-20-9]	$C_9H_{21}N$		NCCCCCCCCC	10.6[a]	143.27	3.29 / -
n-Decylamine [2016-57-1]	$C_{10}H_{23}N$		NCCCCCCCCCC	10.6[a]	157.3	3.78 / -

Table 3: (Continued)

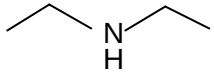
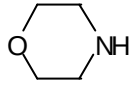
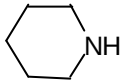
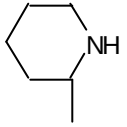
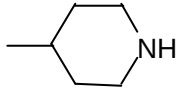
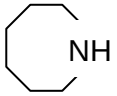
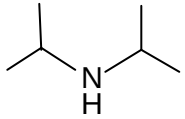
Substance/CAS-No.	**Formula**	**Structures**	**SMILES**	**pK_a**	**MW**	**log K_{ow} est.[f] / exp.**
Secondary amines						
Diethylamine [109-89-7]	$C_4H_{11}N$		N(CC)CC	11.1[a]	73.14	0.81 / 0.58[g]
Morpholine [110-91-8]	C_4H_9NO		O(CCNC1)C1	8.49[c]	87.12	-0.56 / -0.86[j]
Piperidine [110-89-4]	$C_5H_{11}N$		N(CCCC1)C1	11.3[a]	85.15	1.19 / 0.84[g]
2-Methylpiperidine [109-05-7]	$C_6H_{13}N$		N(C(CCC1)C)C1	11.1[a]	99.18	1.61 / -
4-Methylpiperidine [626-58-4]	$C_6H_{13}N$		N(CCC(C1)C)C1	11.1[d]	99.18	1.61 / -
Hexamethyleneimine [111-49-9]	$C_6H_{13}N$		N(CCCCC1)C1	11.1[a]	99.18	1.68 / -
Diisopropylamine [108-18-9]	$C_6H_{15}N$		N(C(C)C)C(C)C	11.1[e]	101.19	1.64 / 1.4[i]

Table 3: (Continued)

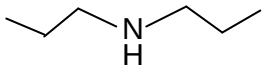
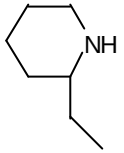
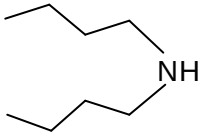
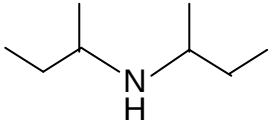
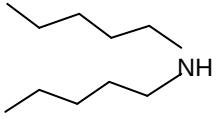
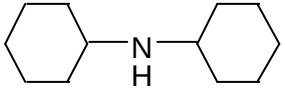
Substance/CAS-No.	**Formula**	**Structures**	**SMILES**	**pK_a**	**MW**	**log K_{ow} est.[f] / exp.**
Secondary amines						
Dipropylamine [142-84-7]	$C_6H_{15}N$		N(CCC)CCC	11.0[a]	101.19	1.79 / 1.67[g]
2-Ethylpiperidine [1484-80-6]	$C_7H_{15}N$		N(C(CCC1)CC)C1	11.1[d]	113.2	2.1 / -
Dibutylamine [111-92-2]	$C_8H_{19}N$		N(CCCC)CCCC	10.9[a]	129.25	2.77 / 2.83[g]
Diisobutylamine [110-58-7]	$C_8H_{19}N$		N(CC(C)C)CC(C)C	11.4[a]	129.25	2.63 / -
Dipentylamine [2050-92-2]	$C_{10}H_{23}N$		N(CCCCC)CCCCC	11.2[a]	157.3	3.76 / -
Dicyclohexylamine [101-83-7]	$C_{12}H_{23}N$		N(C(CCCC1)C1)C(CCCC2)C2	10.4[f]	181.32	4.37 / -

Table 3: (Continued)

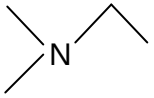
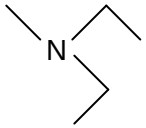
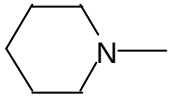
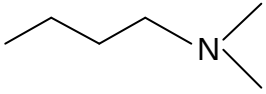
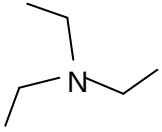
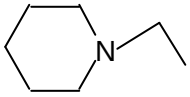
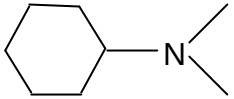
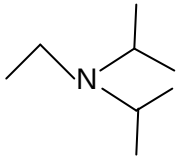
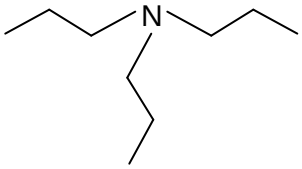
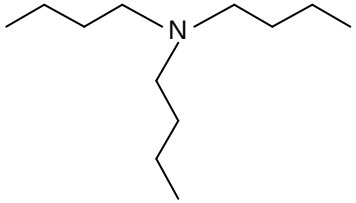
Substance/CAS-No.	**Formula**	**Structures**	**SMILES**	**pK_a**	**MW**	**log K_{ow} est.[f] / exp.**
Tertiary amines						
N,N-Dimethylethylamine [598-56-1]	$C_4H_{11}N$		N(CC)(C)C	10.2[a]	73.13	0.53 / 0.7[g]
N,N-Diethylmethylamine [616-39-7]	$C_5H_{13}N$		N(CC(CC)C	10.2[d]	87.15	1.02 / -
1-Methylpiperidine [626-67-5]	$C_6H_{13}N$		N(CCCC1(C1)C	10.1[a]	99.18	1.4 / 1.3[g]
N,N-Dimethylbutylamine [927-62-8]	$C_6H_{15}N$		N(CCCC)(C)C	10.2[a]	101.19	1.51 / 1.7[g]
Triethylamine [121-44-8]	$C_6H_{15}N$		N(CC)(CC)CC	10.8[e]	101.19	1.51 / 1.45[g]
1-Ethylpiperidine [766-09-6]	$C_7H_{15}N$		N(CCCC1)(C1)CC	10.1[d]	87.17	1.33 / 1.49[g]

Table 3: (Continued)

Substance/CAS-No.	**Formula**	**Structures**	**SMILES**	**pK_a**	**MW**	**log K_{ow} est.[f] / exp.**
Tertiary amines						
N,N-Dimethylcyclohexylamine [98-94-2]	$C_8H_{17}N$		N(C(CCCC1)C1)(C)C	10.6[d]	127.23	2.31 / -
N,N-Diisopropylethylamine [7087-68-5]	$C_8H_{19}N$		N(C(C)C)(C(C)C)CC	10.2[d]	129.14	2.35 / -
Tripropylamine [102-69-2]	$C_9H_{21}N$		N(CCC)(CCC)CCC	10.7[a]	143.27	2.99 / 2.79[g]
Tributylamine [102-82-9]	$C_{12}H_{27}N$		N(CCCC)(CCCC)CCCC	10.9[e]	185.36	4.46 / -

[a] experimental pK_a-values (Perrin, 1965)
[b] experimental pK_a-values (Perrin and Fabian, 1996)
[c] experimental pK_a-values (Perrin, 1972)
[d] values were estimated from similar molecules, because no data were available
[e] experimental pK_a-values (Riddick *et al.*, 1986)
[f] estimated values calculated with the programme KowWin 1.90
[g] experimental log K_{ow}-values (Sangster, 1989)
[h] experimental log K_{ow}-values (Hansch and Leo, 1981)
[i] experimental log K_{ow}-values (Abraham *et al.*, 1994)
[j] experimental log K_{ow}-value (Hansch and Leo, 1985)

2.4 Bioconcentration of aliphatic amines

The bioconcentration is defined as the accumulation of chemicals via the waterphase by gills and/or surface of fish or other aquatic animals. To investigate the accumulation potentials of aliphatic amines in the zebrafish eggs the labelled model compound ^{14}C-butylamine (specific activity: 0.1 mCi*mL^{-1} or 55 mCi*mmol^{-1}; Biotrend, Chemikalien GmbH, Köln, Germany) was used. The eggs were obtained according to the method described above (see chapter 2.1.2). Ensenbach (1987) found that the average wet weight of an egg is 0.664 mg (n = 305).

The exposure system is shown in Figure 7. The exposure was performed under static conditions at 26 ± 1°C in a closed basin filled with 500 mL reconstituted water. 1 mgL^{-1} of gentamycinsulfate (Sigma-Aldrich®, Seelze, Germany) was added to prevent bacterial growth. The LC_{50} of gentamycinsulfate for the embryos of zebrafish was greater than 10 mgL^{-1} (Brust, unpublished data, 2001). The oxygen saturation and the pH were measured prior to the exposure and were 92 % and 7.4, respectively. The water was aerated and waste air was passed through an empty bottle to collect evaporating water, through a bottle with toluene (150 mL) to detect evaporated amines, and through a bottle with KOH solution (10 %, w/v; 150 mL) to collect $^{14}CO_2$. The duration of the exposure was 48 h. Three subsamples of eggs (n = 7) for examining the kinetics of uptake and water samples were taken at defined intervals during the exposure (t_0, $t_{½}$; t_1, t_3; t_8; t_{12} and t_{24}).

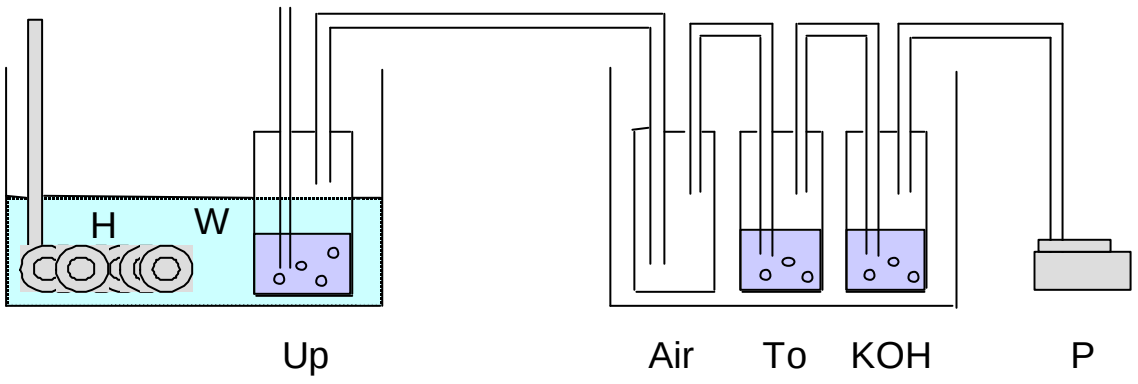


Figure 7: Exposure system for investigating the bioconcentration of chemicals in the eggs of *Danio rerio*. The uptake basin (Up) was placed in a water bath (W) to keep the temperature constant using a heating system (H; Ministat, Huber, Offenburg-Elgersweier, Germany). A suction pump (P) was used to aerate the water in the uptake basin, according to the low pressure principle. (Air = empty bottle; To = bottle with toluene; KOH = bottle with KOH)

The eggs were collected with a pipette and the adherent water was removed with a paper towel (Kimwipes®, Merck, Darmstadt, Germany). The eggs were disintegrated with 1 mL of soluene 350 (Packard Instruments, Frankfurt, Germany) and after one hour 10 mL of Hionic Flour used as scintillation fluid (Packard, Dreieich, Germany) were added. At each sampling time 1 mL water was taken and 10 mL Roteszint 2211 (Roth, Karlsruhe, Germany) were added to the sample.

The amount of ^{14}C in all samples was measured using a Fluid-Scintillation-Counter (TriCarb 2550 TR/AB, Canberra-Packard GmbH, Frankfurt, Germany).

To describe the uptake of chemicals into organisms the standard one-compartment, first-order kinetics model was used (Nagel, 1988). The course of uptake is described by equation 2.

The bioconcentration factor (BCF) was calculated by the steady state approach, which is defined as the steady state between uptake and elimination, using equation 3.

$$(2) \qquad C_o = C_w * \frac{k_1}{k_2} * (1 - e^{-k_2 * t})$$

$$(3) \qquad BCF = \frac{C_o}{C_w} = \frac{k_1}{k_2} \qquad \text{(steady state)}$$

where C_o represents the concentration of the chemical in the organism at time t, C_w the concentration in water, k_1 the uptake rate constant and k_2 the elimination rate constant.
The metabolism of ^{14}C-butylamine was not studied within this experiment.

2.5 Molecular descriptors

Physicochemical and structural parameters for the amines were compiled on features regarded as being relevant for their effects. The descriptors used for the analysis are listed in Table 4.

The lipophilicity is commonly employed as a measure to model partition processes and is given as the logarithm of the octanol-water partition coefficient. In order to describe the lipophilicity log K_{ow} values were calculated with the programme KowWin 1.90 [log K_{ow} (estimated)]. Published data were used for the measured log K_{ow} [log K_{ow} (experimental)] (Table 3, chapter 2.3). Estimated values were used for compounds were an experimental log K_{ow} was not available. The estimated values were then adjusted to known experimental values of similar compounds, to make them better comparable with the experimental data using equation 4:

(4) $$log\ K_{ow}\ (adj.\ A) = log\ K_{ow}\ (est.\ A) + log\ K_{ow}\ (exp.\ B) - log\ K_{ow}\ (est.\ B)$$

(adj. = adjusted; est. = estimated; exp. = experimental)

where *A* is the compound without experimental log K_{ow} and *B* the compound with known experimental log K_{ow}. For example, for isopentylamine (IPeA) only an estimated log K_{ow} and for pentylamine (PeA) a estimated log K_{ow} as well as an experimental log K_{ow} were available. Using the equation 4 the adjusted log K_{ow} of isopentylamine is:

$$log\ K_{ow}\ (adj.\ IPeA) = 1.25\ (est.\ IPeA) + 1.49\ (exp.\ PeA) - 1.33\ (est.\ PeA) = 1.41$$

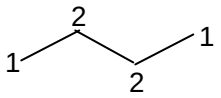
Connectivity indices χ of different order were used as topological descriptors and calculated with the programme PropertEst (1996). They reflect the connectivity between atoms in a molecule based on graph theory (Basak, 1990). The connectivity indices refer only to the two-dimensional formulas, whereas steric features are not considered (Kubinyi, 1993). The calculation of such indices is relatively simple. Using the equation 5 the connectivity index of the first order ($^1\chi$) can be calculated:

(5) $$^1\chi = \Sigma (d_i d_j)^{-\frac{1}{2}}$$

where d corresponds to the d -value of each atom in the molecule.

As an example the calculation of the $^1\chi$ is shown exemplary for propylamine:

n propylamine



$^1\chi$: $2 * [(1*2)^{-1/2}] + (2 * 2)^{-1/2} = 1.91$

Geometrical descriptors used are: molar volume, solvent accessible surface area, solvent accessible volume and the molecule diameter. The shape and size of molecules play an important rule in processes related to the fate, effects and behaviour of environmental contaminants such as size-limited diffusion and the fit to target sites (Nendza, 1998).

The molar volume can be calculated using the quotient of the molecular weight and the density of the respective substance. The solvent-accessible volume (SAVOL) and surface area (SASA) depend on the size of the solvent molecule which in general is water (Nendza, 1998). The SASA can be defined as the locus of the centre of a spherical solvent which roll over the van der Waals surface of the solute (Nendza, 1998). Based on the three-dimensional structures, preferably with their geometry optimised by force field methods, diameters of molecules along various axes can be calculated (Schüürmann, 1990a). The effective diameter (D_{eff}) conforms to the minimal pore width required for the passage through a barrier such as a membrane (Nendza, 1998). They were also calculated with the programme PropertEst (1996).

For calculation of the quantum chemical (e_{HOMO}, e_{LUMO}, dipole moment, hardness, heat of formation, atomic charges) and geometrical descriptors the two-dimensional structures were pre-optimised using the model builder PRXBLD and the resulting three-dimensional structures were optimised with MOPAC 6.0 (Keywords: GNORM=0.1, EF, ESP) using the PM3 Hamiltonian (Stewart, 1989).

Quantum-chemical and geometrical (three-dimensional) descriptors were derived from the MOPAC results (Müller, 1993). Atomic charges were calculated as ESP-charges (electrostatic potential charges, Besler *et al.*, 1990), which gain the best results as compared with simple net atomic charges. Additionally, atomic charges for the nitrogen atom were calculated empirically using the method from Gasteiger and Marsili (1980).

All physicochemical parameters were calculated for the amines without consideration of potential metabolites. The values of all descriptors are compiled in Table A1 (see Appendix).

Table 4: Molecular descriptors used in the analysis

Parameter	**Description**
log K_{ow} (est.)	lipophilicity, estimated log K_{ow} [(a)]
log K_{ow} (exp.)	lipophilicity, experimental log K_{ow}
log K_{ow} (adj.)	lipophilicity, adjusted estimated log K_{ow} (see equation 4)
pK_a	acid-base constant
e_{HOMO}	energy of the highest occupied molecular orbital
e_{LUMO}	energy of the lowest unoccupied molecular orbital
DIFF	e_{HOMO} - e_{LUMO}
Hardness	hardness = $(-e_{HOMO} + e_{LUMO}) / 2$ [(b)]
EN	electronegativity = $(-e_{HOMO} - e_{LUMO}) / 2$ [(b)]
HOF	heat of formation
Dipol	dipole moment
D_{max}	maximum diameter [(c)]
D_{eff}	effective diameter [(c)]
D_{min}	minimum diameter [(c)]
SASA	solvent accessible surface area [(d)]
SAVOL	solvent accessible volume [(d)]
V^+	potential of the positive atomic charges [(e)] [(f)]
V^-	potential of the negative atomic charges [(e)] [(f)]
V^{tot}	potential of the total atomic charges [(e)] [(f)]
Q^+_{max}	maximum positive atomic charge [(e)]
Q^-_{max}	maximum negative atomic charge [(e)]
Q_{tot}	maximum atomic charge (absolute) [(e)]
Q_{av}	average of absolute atomic charges [(e)]
H^+_{max}	maximum positive charge on hydrogen atom [(e)]
MW	molecular weight
$^0\chi$	connectivity index of zero order
$^1\chi$	connectivity index of first order
$^2\chi$	connectivity index of second order
$^3\chi^p$	connectivity index of third order (path)
$^3\chi^c$	connectivity index of third order (cluster)
$^0\chi^v$	connectivity index of zero order (valence corrected)
$^1\chi^v$	connectivity index of first order (valence corrected)
$^2\chi^v$	connectivity index of second order (valence corrected)
$^3\chi^{v,p}$	connectivity index of third order [valence corrected (path)]
MOLVOL	molar volume [(g)]
N-ESP	partial charge at N-atom (ESP calculation)
N-Gasteiger	partial charge at N-atom according to Gasteiger [(h)]

[(a)] KowWin (Version 1.90)
[(b)] Pearson, 1986
[(c)] CROSS, 1996
[(d)] GEPOL 93
[(e)] all charges dependent parameters except N-Charge and N-Gasteiger were derived from MOPAC/ESP-charges
[(f)] Schüürmann, 1990b
[(g)] PropertEst, 1996
[(h)] Gasteiger and Marsili, 1980

2.6 Calculations and Statistics

The lethal endpoints were used to calculate median lethal concentration values (48-h LC_{50}) using the Probit-Transformation (Litchfield and Wilcoxon, 1949) and a 95 % confidence interval is given.

The bioconcentration factor were calculated by a non-linear regression model using Statistica© 5.0 for Windows (StatSoft, Inc.).

To explain the relationship between the toxicity and the molecular descriptors simple and multiple linear regressions were used (Win STAT® for Windows® Excel, version 2000.1). To avoid chance correlations between molecular descriptors the intercorrelation should not exceed r = 0.6. The median lethal concentration is given as log ($1/LC_{50}$) which is the logarithm of the inverse of the 48 h 50% mortality concentration ($\mu molL^{-1}$) for the embryos of zebrafish. The median lethal dose is given as the log ($1/LD_{50}$) which is the logarithm of the inverse of the 48 h 50% mortality dose ($\mu molkg^{-1}$] for the embryos of zebrafish.

Simple and multiple linear regressions were cross-validated by using either "leave several out" (LSO) or "leave one out" (LOO) procedures. The cross-validation is a reasonable method to check statistical models for an internal validation (Geladi and Kowalski, 1986; Cramer *et al.*, 1988). The principle is that the data set is divided into groups and the model is recalculated without the data of the other groups. Predictions are obtained for omitted compounds and compared with the actual data. Using the predicted error sum of squares divergences can be quantified and transformed to the dimensionless term Q^2, which represents the cross-validated explained variance and is the counterpart of the explained variance R^2. If the squared correlation coefficient (Q^2) or crossvalidation coefficient is greater than 0.5, then the regression can be regarded as valid using predicted residuals. The greater the Q^2 the better the coherency between the parameters.

If necessary, a bilinear regression model (Kubinyi, 1977) was used to describe the relationship between parameters using equation 6:

(6) $$log(1/LC_{50}) = a*log\ K_{ow} - b*log(b*K_{ow} + 1) - c.$$

3 RESULTS AND DISCUSSION

3.1 Toxicity of aliphatic amines – a literature survey

In the following all available toxicity data from literature were compiled. In Table 5 the number of available toxicity data for the aliphatic amines investigated in this study are summarised. However, data derived from fish in long-time exposure studies are not included.

Table 5: Number of toxicity data found in the literature for the aliphatic amines investigated in this study. Data for fish and cladoceran are subdivided into the different test durations.

Substance group	**Algae** [a]	**Cladocera**[b]		**Fish**			**Rat**[f]	S **Total**
		24h	**48h**	**24h**[c]	**48h**[d]	**96h**[e]		
Primary amines	2	1	9	6	4	11	8	41
Secondary amines	4	3	3	6	1	7	10	34
Tertiary amines	0	0	1	3	1	1	6	12

[a] *Selenastrum capricornutum*
[b] *Daphnia magna*
[c] data derived from *Semotilus atromaculatus*, given as LC_{100}
[d] data derived from *Oryzias latipes*, given as LC_{50}
[e] data derived from *Pimephales promelas*, *Oncorhynchus mykiss*, and/or *Danio rerio,* given as LC_{50}
[f] data derived from rat oral toxicity, given as LD_{50}

The Table 5 shows that most toxicity data are available for the group of primary amines (47 %). In some cases one compound was tested using several fish species. The number of toxicity data found for secondary amines corresponds to 34 % and for the tertiary amines only to 12 %. No toxicity data were found for the tertiary amines in the case of algae.

Nevertheless, these data can be used to compare the toxicity among the fish species, if the test designs are comparable. Furthermore, for the cladoceran as well as for the algae simple toxicity relationships can be predicted using the lipophilicity as single descriptor and can be compared with the model equations derived from the embryo tests with *Danio rerio*. Similarly, the lethal-dose-data (LD_{50}) of the mammalian toxicity can be used to compare them with predicted LD_{50} values of the embryo *Danio rerio*. In Table 6 some data for several fish species, for the caldoceran *Daphnia magna* and for the alga *Selenastrum capricornutum* are listed. Additionally, data for rat oral toxicity are presented.

Table 6: Toxicity data of primary, secondary and tertiary aliphatic amines for fish, cladocera, algae and mammals compiled from literature. Data including test duration, endpoints (LC_x; LD_{50}), and the corresponding reference. Information about test conditions were only included for the aquatic tests.

Substance	Organism	Toxicity	Test conditions	Reference
n-Propylamine	Fish:			
	Oryzias latipes	LC_{50} (48h) = 16,918 µmolL^{-1}	static, 25°C; pH = neutralised	(Tonogai *et al.*, 1982)
	Pimephales promelas	LC_{50} (96h) = 5,211 µmolL^{-1}	flow-through, 25±1°C	(Brooke *et al.*, 1984)
	Semotilus atromaculatus	LC_{100} (24h) = 1,015 µmolL^{-1}	static, 15 – 21°C	(Gillette *et al.*, 1952)
	Invertebrates:			
	Daphnia magna	EC_{50} (48h) = >1,692 µmolL^{-1}	static, 20±1°C; pH = 7.8±0.1	(Pedersen *et al.*, 1998)
	Mammalia:			
	Rat (oral)	LD_{50} = 6,260 – 9,643 µmolkg^{-1}		(Greim *et al.*, 1998)
Isopropylamine	Fish:			
	Danio rerio	LC_{50} (96h) = 91,200 µmolL^{-1} [ET][a]	static, 25±1°C; pH = 8.0	(Groth *et al.*, 1993)
	Oryzias latipes	LC_{50} (48h) = 16,918 µmolL^{-}	static, 25°C; pH = neutralised	(Tonogai *et al.*, 1982)
	Semotilus atromaculatus	LC_{100} (24h) = 1,353 µmolL^{-1}	static, 15 – 21°C	(Gillette *et al.*, 1952)
	Invertebrates:			
	Daphnia magna	EC_{50} (48h) = 1,550 µmolL^{-1}	static, 20±1°C; pH = unadjusted	(Chester *et al.*, 1992)
	Algae:			
	Selenastrum capricornutum	IC_{50} (96h) = < 1057 µmolL^{-1}	semistatic, 20±1°C; pH = unadjusted	(Chester *et al.*, 1992)
	Mammalia:			
	Rat (oral)	LD_{50} = 7,520 µmolkg^{-1}		(Greim *et al.*, 1998)
n-Butylamine	Fish:			
	Oryzias latipes	LC_{50} (48h) = 13,672 µmolL^{-1}	static, 25°C; pH = neutralised	(Tonogai *et al.*, 1982)
	Pimephales promelas	LC_{50} (96h) = 3,664 µmolL^{-1}	flow-through, 25°C	(Broderius *et al.*, 1995)
	Semotilus atromaculatus	LC_{100} (24h) = 957 µmolL^{-1}	static, 15 – 21°C	(Gillette *et al.*, 1952)
	Invertebrates:			
	Daphnia magna	EC_{50} (48h) = >1,692 µmolL^{-1}	static, 20±1°C; pH = 7.8±0.1	(Pedersen *et al.*, 1998)
	Mammalia:			
	Rat (oral)	LD_{50} = 5,004 µmolkg^{-1}		(Jäckel and Klein, 1991)

[a] [ET = embryotest]

Table 6: (Continued)

Substance	Organism	Toxicity	Test conditions	Reference
Isobutylamine	Fish:			
	Semotilus atromaculatus	LC_{100} (24h) = 820 µmolL^{-1}	static, 15 – 21°C	(Gillette *et al.*, 1952)
	Mammalia:			
	Rat (oral)	LD_{50} = 3,063 µmolkg^{-1}		(Merck, Safety Data Sheet)
sec-Butylamine	Fish:			
	Semotilus atromaculatus	LC_{100} (24h) = 820 µmolL^{-1}	static, 15 – 21°C	(Gillette *et al.*, 1952)
	Pimephales promelas	LC_{50} (96h) = 3,760 µmolL^{-1}	flow-through, 25°C; pH = 7.7	(Newsome *et al.*, 1991)
	Mammalia:			
	Rat (oral)	LD_{50} = 7,451 µmolkg^{-1}		(Greim *et al.*, 1998)
n-Pentylamine	Fish:			
	Pimephales promelas	LC_{50} (96h) = 2,031 µmolL^{-1}	flow-through, 25°C	(Broderius *et al.*, 1995)
	Semotilus atromaculatus	LC_{100} (24h) = 574 µmolL^{-1}	static, 15 – 21°C	(Gillette *et al.*, 1952)
	Invertebrates:			
	Daphnia magna	EC_{50} (48h) = 646 µmolL^{-1}	semistatic, 20±1°C; pH = 7.8±0.1	(Pedersen *et al.*, 1998)
Cyclohexylamine	Fish:			
	Danio rerio	LC_{50} (96h) = 4,739 µmolL^{-1}	static, 22°C; pH = 7.5	(Wellens, 1982)
	Oncorhynchus mykiss	LC_{50} (96h) = 444 µmolL^{-1}	static, 15°C; pH = 7.4	(Calamari *et al.*, 1980)
	Invertebrates:			
	Daphnia magna	EC_{50} (24h) = 494 µmolL^{-1}	static, 20±1°C	(Calamari *et al.*, 1980)
	Algae:			
	Selenastrum capricornutum	IC_{50} (96h) = 202 µmolL^{-1}	static, 20±1°C	(Calamari *et al.*, 1980)
	Mammalia:			
	Rat (oral)	LD_{50} = 1,573 – 6,191 µmolkg^{-1}		(Greim *et al.*, 1998)

Table 6. (Continued)

Substance	Organism	Toxicity	Test conditions	Reference
n-Hexylamine	Fish:			
	Pimephales promelas	LC_{50} (96h) = 559 µmolL^{-1}	flow-through, 25°C	(Broderius *et al.*, 1995)
	Invertebrates:			
	Daphnia magna	EC_{50} (48h) = 85 µmolL^{-1}	semistatic, 20±1°C; pH = 7.8±0.1	(Pedersen *et al.*, 1998)
	Mammalia:			
	Rat (oral)	LD_{50} = 6,621 µmolkg^{-1}		(Jäckel and Klein, 1991)
n-Heptylamine	Fish:			
	Pimephales promelas	LC_{50} (96h) = 189 µmolL^{-1}	flow-through, 25±1°C; pH = 7.7	(Newsome *et al.*, 1991)
	Invertebrates:			
	Daphnia magna	EC_{50} (48h) = 82 µmolL^{-1}	semistatic, 20±1°C; pH = 7.8±0.1	(Pedersen *et al.*, 1998)
n-Octylamine	Fish:			
	Pimephales promelas	LC_{50} (96h) = 40 µmolL^{-1}	flow-through, 25±1°C; pH = 7.7	(Geiger *et al.*, 1988)
	Invertebrates:			
	Daphnia magna	EC_{50} (48h) = 15 µmolL^{-1}	semistatic, 20±1°C; pH = 7.8±0.1	(Pedersen *et al.*, 1998)
n-Nonylamine	Fish:			
	Pimephales promelas	LC_{50} (96h) = 15 µmolL^{-1}	flow-through, 25±1°C; pH = 7.7	(Geiger *et al.*, 1990)
	Invertebrates:			
	Daphnia magna	EC_{50} (48h) = 11 µmolL^{-1}	semistatic, 20±1°C; pH = 7.8±0.1	(Pedersen *et al.*, 1998)
n-Decylamine	Fish:			
	Pimephales promelas	LC_{50} (96h) = 7 µmolL^{-1}	flow-through, 25±1°C; pH = 7.7	(Newsome *et al.*, 1991)
	Invertebrates:			
	Daphnia magna	EC_{50} (48h) = 4 µmolL^{-1}	semistatic, 20±1°C; pH = 7.8±0.1	(Pedersen *et al.*, 1998)
	Mammalia:			
	Rat (oral)	LD_{50} = 1,780 µmolkg^{-1}		(Greim *et al.*, 1998)

Table 6: (Continued)

Substance	Organism	Toxicity	Test conditions	Reference
Diethylamine	Fish:			
	Oryzias latipes	LC_{50} (48h) = 13,672 µmolL^{-1}	static, 25°C; pH = neutralised	(Tonogai *et al.*, 1982)
	Oncorhynchus mykiss	LC_{50} (96h) = 342 µmolL^{-1}	static, 15°C; pH = 7.4	(Calamari *et al.*, 1980)
	Pimephales promelas	LC_{50} (96h) = 11,690 µmolL^{-1}	flow-through, 25±1°C	(Brooke *et al.*, 1984)
	Semotilus atromaculatus	LC_{100} (24h) = 13,672 µmolL^{-1}	static, 15 – 21°C	(Gillette *et al.*, 1952)
	Invertebrates:			
	Daphnia magna	EC_{50} (24h) = 2,242 µmolL^{-1}	static, 20±1°C	(Calamari *et al.*, 1980)
	Algae:			
	Selenastrum capricornutum	IC_{50} (96h) = 273 µmolL^{-1}	static, 20±1°C	(Calamari *et al.*, 1980)
	Mammalia:			
	Rat (oral)	LD_{50} = 7,383 µmolkg^{-1}		(Greim *et al.*, 1998)
Dipropylamine	Fish:			
	Semotilus atromaculatus	LC_{100} (24h) = 593 µmolL^{-1}	static, 15 – 21°C	(Gillette *et al.*, 1952)
	Mammalia:			
	Rat (oral)	LD_{50} = 4,546 – 9,191 µmolkg^{-1}		(Greim *et al.*, 1998)
Diisopropylamine	Fish:			
	Danio rerio	LC_{50} (96h) = 1,878 µmolL^{-1}	semistatic, 23±2°C	(Canton *et al.*, 1984)
	Oncorhynchus mykiss	LC_{50} (96h) = 366 µmolL^{-1}	static, 15°C; pH = 7.4	(Calamari *et al.*, 1980)
	Semotilus atromaculatus	LC_{100} (24h) = 593 µmolL^{-1}	static, 15 – 21°C	(Gillette *et al.*, 1952)
	Invertebrates:			
	Daphnia magna	EC_{50} (48h) = 4427 µmolL^{-1}	static, 19±1°C	(Hermens *et al.*, 1984b)
	Algae:			
	Selenastrum capricornutum	IC_{50} (96h) = 198 µmolL^{-1}	static, 20±1°C	(Calamari *et al.*, 1980)
	Mammalia :			
	Rat (oral)	LD_{50} = 4,941 µmolkg^{-1}		(Jäckel and Klein, 1991)

Table 6: (Continued)

Substance	Organism	Toxicity	Test conditions	Reference
Dibutylamine	Fish:			
	Oncorhynchus mykiss	LC_{50} (96h) = 286 µmolL^{-1}	static, 15°C; pH = 7.4	(Calamari *et al.*, 1980)
	Semotilus atromaculatus	LC_{100} (24h) = 464 µmolL^{-1}	static, 15 – 21°C	(Gillette *et al.*, 1952)
	Invertebrates:			
	Daphnia magna	EC_{50} (24h) = 1,238 µmolL^{-1}	static, 20±1°C	(Calamari *et al.*, 1980)
	Algae:			
	Selenastrum capricornutum	IC_{50} (96h) = 147 µmolL^{-1}	static, 20±1°C	(Calamari *et al.*, 1980)
	Mammalia:			
	Rat (oral)	LD_{50} = 1,462 – 4,255 µmolkg^{-1}		(Greim *et al.*, 1998)
Diisobutylamine	Fish:			
	Semotilus atromaculatus	LC_{100} (24h) = 310 µmolL^{-1}	static, 15 – 21°C	(Gillette *et al.*, 1952)
	Invertebrates:			
	Daphnia magna	EC_{50} (48h) = 271 µmolL^{-1}	semistatic, 20±1°C; pH = 7.8±0.1	(Danish EPA, 1999)
	Mammalia:			
	Rat (oral)	LD_{50} = 1,996 µmolkg^{-1}		(Acros Organics, Safety Data Sheet)
Dipentylamine	Fish:			
	Semotilus atromaculatus	LC_{100} (24h) = 127 µmolL^{-1}	static, 15 – 21°C	(Gillette *et al.*, 1952)
	Mammalia:			
	Rat (oral)	LD_{50} = 1,716 µmolkg^{-1}		(Acros Organics, Safety Data Sheet)
Morpholine	Fish:			
	Danio rerio	LC_{50} (96h) = 11,478 µmolL^{-1}	static, 22°C; pH = 7.5	(Wellens, 1982)
	Oncorhynchus mykiss	LC_{50} (96h) = 4,362 µmolL^{-1}	static, 15°C; pH = 7.4	(Calamari *et al.*, 1980)
	Invertebrates:			
	Daphnia magna	EC_{50} (24h) = 1366 µmolL^{-1}	static, 20±1°C	(Calamari *et al.*, 1980)
	Algae:			
	Selenastrum capricornutum	IC_{50} (96h) = 321 µmolL^{-1}	static, 20±1°C	(Calamari *et al.*, 1980)
	Mammalia:			
	Rat (oral)	LD_{50} = 12,052 µmolkg^{-1}		(Greim *et al.*, 1998)

Table 6: (Continued)

Substance	Organism	Toxicity	Test conditions	Reference
Piperidine	Mammalia:			
	Rat (oral)	LD_{50} = 5,285 – 6,107 µmolkg^{-1}		(Greim *et al.*, 1998)
Hexamethyleneimine	Mammalia:			
	Rat (oral)	LD_{50} = 4,134 µmolkg^{-1}		(Merck, Safety Data Sheet)
Dicyclohexylamine	Invertebrates:			
	Daphnia magna	EC_{50} (48h) = 387 µmolL^{-1}	semistatic, 20±1°C; pH = 7.8±0.1	(Danish EPA, 1999)
	Mammalia:			
	Rat (oral)	LD_{50} = 1,103 – 2,057 µmolkg^{-1}		(Greim *et al.*, 1998)
Triethylamine	Fish:			
	Oryzias latipes	LC_{50} (48h) = 7,115 µmolL^{-1}	semistatic, 25±1°C; pH = 8.4±0.2	(Tonogai *et al.*, 1982)
	Semotilus atromaculatus	LC_{100} (24h) = 791 µmolL^{-1}	static, 25°C; pH = neutralised	(Gillette *et al.*, 1952)
	Mammalia:		static, 15 – 21°C	
	Rat (oral)	LD_{50} = 4,546 µmolkg^{-1}		(Greim *et al.*, 1998)
Tripropylamine	Fish:			
	Pimephales promelas	LC_{50} (96h) = 355 µmolL^{-1}	flow-through, 25±1°C; pH = 7.8	(Geiger *et al.*, 1986)
	Semotilus atromaculatus	LC_{100} (24h) = 489 µmolL^{-1}	static, 15 – 21°C	(Gillette *et al.*, 1952)
	Mammalia:			
	Rat (oral)	LD_{50} = 503 µmolkg^{-1}		(Merck, Safety Data Sheet)

Table 6: (Continued)

Substance	**Organism**	**Toxicity**	**Test conditions**	**Reference**
Tributylamine	Fish:			
	Semotilus atromaculatus	LC_{100} (24h) = 216 µmolL^{-1}	static, 15 – 21°C	(Gillette *et al.*, 1952)
	Invertebrates:			
	Daphnia magna	EC_{50} (48h) = 43 µmolL^{-1}	semistatic, 20±1°C; pH = 7.5	(Pedersen, 2000)
	Mammalia:			
	Rat (oral)	LD_{50} = 2,913 µmolkg^{-1}		(Jäckel and Klein, 1991)
Dimethylethylamine	Mammalia:			
	Rat (oral)	LD_{50} = 8,287 µmolkg^{-1}		(Greim *et al.*, 1998)
N,N-Dimethylcyclohexylamine	Mammalia:			
	Rat (oral)	LD_{50} = 2,735 – 5,109 µmolkg^{-1}		(Greim *et al.*, 1998)
1-Ethylpiperidine	Mammalia:			
	Rat (oral)	LD_{50} = 3,212 µmolkg^{-1}		(Merck, Safety Data Sheet)

3.2 The *Danio rerio* toxicity assays

3.2.1 Effects of basicity on the embryos of *Danio rerio*

Prior to embryo toxicity testing with aliphatic amines the effects of basicity were investigated adjusting pH´s of 8.0 to 12.0 using a 0.1 n NaOH – solution. After 48 h neither lethal nor sublethal effects were observed at pH's up to 10.5 (Table 7). At a pH of 11.0 one coagulated embryo was found after 5 h and further two coagulated embryos after 24 h. No further coagulated embryos could be observed until 48 h. In the solutions with a pH of 11.5 and 12.0 all embryos coagulated within the first 5 h of their development. Sublethal effects could not be observed within this test.

Table 7: Lethal effects of basicity on the embryos of zebrafish *Danio rerio* after 5h, 24h, and 48h.

Mortality	**pH-values**								
[%]	**8.0**	**8.5**	**9.0**	**9.5**	**10.0**	**10.5**	**11.0**	**11.5**	**12.0**
5 h	0	0	0	0	0	0	5	100	100
24 h	0	0	0	0	0	0	15	100	100
48 h	0	0	0	0	0	0	15	100	100

Further the of heart beat frequency was counted after 48h of exposure and no significant differences were found between the control and the treatments of pH 8.0 to 11.0 (Figure 8) (one-way ANOVA, $\alpha = 0.05$; $F = 1.25$; $p = 0.276$).

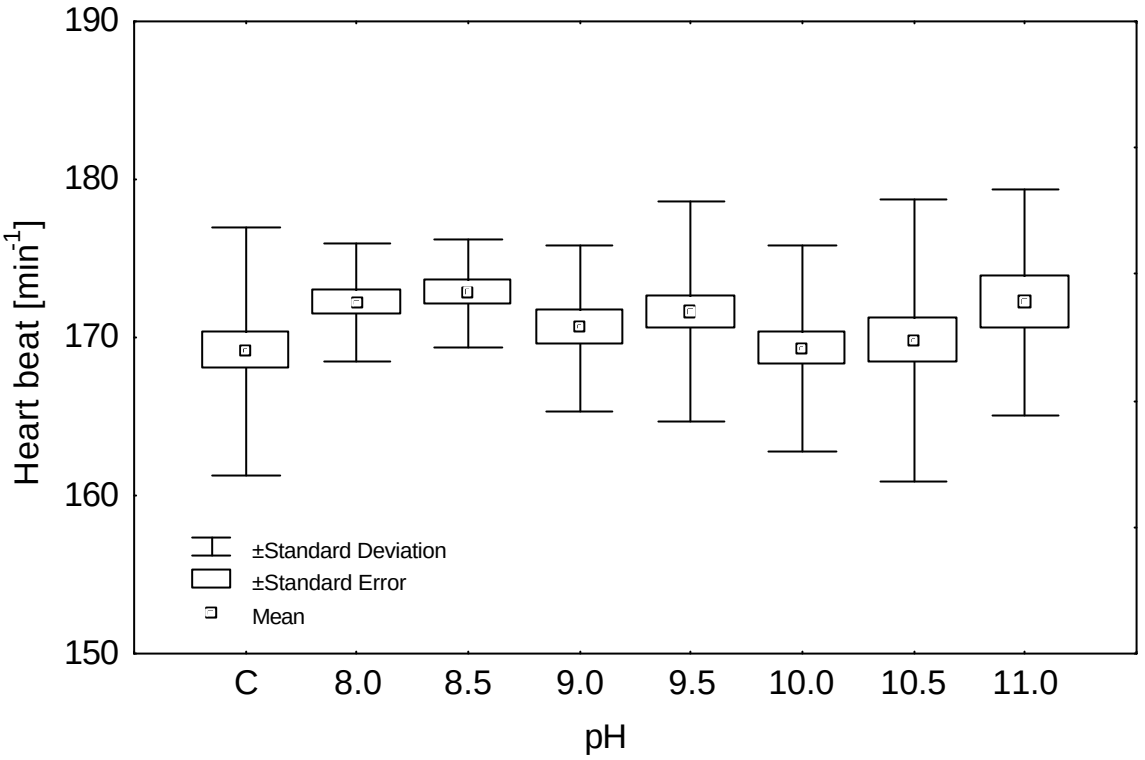


Figure 8: Heart beat frequency of embryos of *Danio rerio* exposed to solutions of different pH´s after 48h. (C = Control [pH = 7.6])

Based on these results a 100 % mortality due to the basicity of the amines can be excluded, if the pH-values does not exceed 11.0 at the beginning of the exposure. Furthermore, pH adjusting was not necessary due to the tolerance of the embryos up to pH-values of 10.5 (see chapter 3.2.1). This observation was in a good agreement with investigations by Johansson *et al.* (1973) and Hermann (1993). They observed a pH tolerance of the zebrafish embryos up to 9.0 and 10.0, respectively.

3.2.2 Effects of aliphatic amines on the embryos of *Danio rerio*

3.2.2.1 Test conditions

The test concentrations, the oxygen concentrations and pH's in the control and in the treatments of all tested amines at the beginning of the exposure (t_0) are summarised in Table 8. The oxygen concentrations in the control and the treatments were in all cases higher than 7 mgL^{-1}. The pH – values of the control treatments ranged between 7.2 and 7.8. As mentioned, in the tested treatments pH – values of > 8 were measured. The highest pHs were measured in the highest substance concentrations but never exceeded 11.2. Due to preliminary results effects of high pH - values on the zebrafish embryos which leading to a 100 % mortality in this pH - range could be excluded (see Table 7, chapter 3.2.1).

Table 8: Range of test concentrations (mgL^{-1}), oxygen concentration (mgL^{-1}) and the pH–values in the control and in the treatments (given as range) of the amines in the treatments at the start of the exposure (t_0).

Substance	**Concentrations**	**O_2**		**pH**	
		Control	**Treatments**	**Control**	**Treatments**
Primary amines					
n-Propylamine	38.5 – 110	8.12	7.90 – 8.18	7.39	10.58 – 11.03
Isopropylamine	52.1 – 200	8.5	7.45 – 7.92	7.68	10.54 – 11.02
n-Butylamine	20.8 – 80	9.03	8.59 – 8.93	7.58	10.26 – 10.91
sec-Butylamine	39 – 150	8.49	8.47 – 8.71	7.48	10.46 – 11.02
Isobutylamine	19.8 – 100	8.47	7.94 – 8.35	7.63	10.25 – 10.98
n-Pentylamine	26.3 – 75	8.46	7.98 – 8.36	7.36	10.31 – 10.86
Isopentylamine	29.8 – 85	8.28	7.51 – 8.01	7.62	10.37 – 10.82
Cyclohexylamine	35 – 100	8.98	8.52 – 8.92	7.39	10.42 – 10.91
n-Hexylamine	24.5 – 70	7.96	7.57 – 7.81	7.56	10.16 – 10.72
n-Heptylamine	17.5 - 50	9.08	8.56 – 8.94	7.43	9.97 – 10.57
n-Octylamine	21 - 60	7.89	7.66 – 7.80	7.43	10.02 – 10.55
n-Nonylamine	10.8 – 30.8	7.64	7.12 – 7.45	7.69	9.81 – 10.41
n-Decylamine	2.1 – 8	7.72	7.42 – 7.98	7.52	9.32 – 9.96

Table 8: (Continued)

Substance	**Concentrations**	**O_2**		**pH**	
		Control	**Treatments**	**Control**	**Treatments**
Secondary amines					
Diethylamine	38.5 – 110	8.66	8.15 – 8.41	7.28	10.53 – 11.07
Morpholine	250 – 1638.4	7.67	7.26 – 7.58	7.40	9.79 – 10.29
Piperidine	49 – 140	7.54	7.10 – 7.32	7.80	10.36 – 10.82
2-Methylpiperidine	52.5 – 150	8.75	8.77 – 8.97	7.33	10.58 – 11.09
4-Methylpiperidine	42 – 120	8.86	8.22 – 8.54	7.32	10.47 – 11.10
Hexamethyleneimine	52.5 – 150	9.2	8.58 – 8.89	7.21	10.63 – 11.19
Diisopropylamine	42 – 120	7.61	7.28 – 7.55	7.52	10.08 – 10.56
Dipropylamine	21 – 60	7.64	7.74 – 7.87	7.39	10.44 – 11.05
2-Ethylpiperidine	35 – 100	8.57	8.51 – 8.61	7.70	10.37 – 10.94
Diisobutylamine	35 – 100	9.12	8.03 – 8.67	7.48	10.63 – 11.05
Dibutylamine	28 – 80	8.78	8.54 – 8.69	7.76	10.20 – 10.77
Dipentylamine	28 – 80	7.5	7.26 – 7.49	7.53	10.27 – 10.84
Dicyclohexylamine	19.8 – 100	8.7	7.87 – 8.41	7.56	10.17 – 11.12
Tertiary amines					
N,N-Dimethylethylamine	29.6 – 150	8.6	8.5 – 8.73	7.4	9.88 – 10.61
N,N-Diethylmethylamine	29.6 – 150	8.54	8.44 – 8.57	7.38	10.03 – 10.78
1-Methylpiperidine	27.3 – 78	8.75	8.56 – 8.78	7.43	10.12 – 10.51
N,N-Dimethylbutylamine	19.8 – 100	8.43	8.35 – 8.64	7.34	9.76 – 10.51
Triethylamine	13.7 – 90	9.02	8.43 – 8.98	7.45	9.95 – 10.86
1-Ethylpiperidine	35 – 100	8.89	8.89 – 8.95	7.77	10.46 – 10.78
N,N-Dimethylcyclo-hexylamine	22 – 85	7.89	7.06 – 7.54	7.40	9.84 – 10.66
N,N-Diisopropyl-ethylamine	57.8 – 165	8.59	8.65 – 8.7	7.34	10.57 – 11.08
Tripropylamine	59.3 – 300	9.23	8.76 – 9.08	7.56	10.30 – 10.97
Tributylamine	61 – 400	8.46	7.43 – 8.17	7.53	8.82 – 9.95

The pH of the test medium is a very important factor for basic as well as for acidic substances. The most chemicals released to the environment are generally evaluated with respect to their non-dissiciated form. This neglects the effects of the dissociation on further properties (Nendza, 1998). The dissociation rate of chemicals can affect the uptake and thus,

the bioavailability in an organism. Non-dissociated substances can better diffuse through biological membranes. Therefore, the dose of a non-dissociated chemical in a organism is higher than that of a dissociated (Könemann and Musch, 1981; Schüürmann and Segner, 1994; Kishino and Kobayashi, 1995). In Table 9 the pK_a – values, the percent of ionisation in the amine treatments calculated using the Henderson-Hasselbalch equation (see chapter 2.3) for the amines are presented. Inspite of similar pK_a-values the degree of ionisation differs due to the different pH's as a result of the different test concentrations of the corresponding test substances (see Table 8).

Table 9: pK_a-values and percent of ionisation of the amines in the treatments (as range from the lowest to the highest test concentration) at the beginning of the exposure (t_0). Ionisation was calculated using the Henderson-Hasselbalch equation (chapter 2.3).

Substance	**pK_a**	**Ionisation [%]**
Primary amines		
n-Propylamine	10.7[a]	57 – 32
Isopropylamine	10.6[b]	40 – 20
n-Butylamine	10.8[a]	78 – 44
sec-Butylamine	10.7[c]	50 – 28
Isobutylamine	10.6[a]	74 – 34
n-Pentylamine	10.6[a]	66 – 35
Isopentylamine	10.6[d]	63 – 38
Cyclohexylamine	10.6[c]	60 – 20
n-Hexylamine	10.6[a]	73 – 43
n-Heptylamine	10.7[a]	84 – 57
n-Octylamine	10.7[a]	83 – 59
n-Nonylamine	10.6[a]	86 – 61
n-Decylamine	10.6[a]	95 – 81

Table 9: (Continued)

Substance	**pK_a**	**Ionisation [%]**
Secondary amines		
Diethylamine	11.1[a]	79 – 52
Morpholine	8.49[c]	5 – 2
Piperidine	11.3[a]	90 – 75
2-Methylpiperidine	11.1[a]	77 – 51
4-Methylpiperidine	11.1[d]	81 – 50
Hexamethyleneimine	11.1[a]	75 – 45
Diisopropylamine	11.1[e]	91 – 78
Dipropylamine	11.0[a]	78 – 47
2-Ethylpiperidine	11.1[d]	84 – 59
Diisobutylamine	10.9[a]	65 – 41
Dibutylamine	11.4[a]	93 - 78
Dipentylamine	11.2[a]	89 – 70
Dicyclohexylamine	10.4[a]	63 – 16
Tertiary amines		
N,N-Dimethylethylamine	10.2[a]	68 – 28
N,N-Diethylmethylamine	10.2[d]	60 – 21
1-Methylpiperidine	10.1[a]	49 – 28
N,N-Dimethylbutylamine	10.2[a]	73 – 33
Triethylamine	10.8[e]	88 – 47
1-Ethylpiperidine	10.1[d]	30 – 17
N,N-Dimethylcyclohexylamine	10.6[d]	85 – 47
N,N-Diisopropylethylamine	10.2[d]	30 – 12
Tripropylamine	10.7[a]	72 – 35
Tributylamine	10.9[e]	99 – 94

[a] experimental pK_a-values (Perrin, 1965)
[b] experimental pK_a-values (Perrin and Fabian, 1996)
[c] experimental pK_a-values (Perrin, 1972)
[d] values were taken from similar molecules, because no data were available
[e] experimental pK_a-values (Riddick *et al.*, 1986)

Within the range of the chosen test concentrations the degree of ionisation differed greatly between the aliphatic amines (Table 9). The degree of ionisation is lower in the highest concentrations as compared to the lowest concentrations of the corresponding test substance. Within the group of the primary amines the highest degree of ionisation was found for

heptylamine, octylamine, nonylamine, and decylamine. Within the secondary amines morpholine was the least ionised compounds. The pK_a – value for morpholine is with 8.49 the lowest compared with all amines tested. The most ionised compound within the tertiary amines was tributylamine. This compound is distinguished by the lowest water solubility compared to the other amines. At the beginning of the exposure (t_0) lower pH's were measured as expected from the pK_a of tributylamine. The very high ionisation might be influenced by the difficulties in preparing the test solution.

However, the degree of ionisation was calculated using the pH-values measured at the beginning of the exposure (t_0). After 48 h the pH in the test solutions could not be measured for technical reasons. Within the 48 h period each single well with 2 mL test solution and the exposed embryo acted as a black box. Further, no analytical methods to determine amine concentrations were performed. For these reasons the degree of ionisation calculated in this study seems to be a speculative fact and should therefore not be taken into account to describe structure-toxicity relationships. Nevertheless, the impact of ionisation might explain differences in the toxicity of aliphatic amines.

3.2.2.2 Lethal effects

During the 48 h toxicity tests all validity criteria were fulfilled as recommended in a draft for an OECD Guideline (Nagel, 1998). Neither lethal nor sublethal effects were found in the controls. The toxicity within 48 hours given as LC_{50} - values with the 95 % confidence intervals for the 36 amines tested using the *Danio rerio* toxicity assay (*Dar*T) are presented in Table 10.

Table 10: Observed toxicity of aliphatic amines (LC_{50} and 95 % confidence) using the *Danio rerio* toxicity assay (*Dar*T).

Substance	**LC_{50} [µmolL^{-1}]**
Primary amines	
n-Propylamine	1,339 (1,200 - 1,492)
Isopropylamine	2,531 (2,322 – 2,575)
n-Butylamine	491 (456 – 528)
sec-Butylamine	1,301 (1,150 – 1,472)
Isobutylamine	1,267 (1,093 – 1,610)
n-Pentylamine	354 (221 – 565)
Isopentylamine	678 (591 – 777)
Cyclohexylamine	639 (584 – 698)
n-Hexylamine	418 (381 – 459)
n-Heptylamine	247 (228 – 268)
n-Octylamine	197 (187 – 207)
n-Nonylamine	80 (76 – 85)
n-Decylamine	20 (18 – 22)
Secondary amines	
Diethylamine	1,275 (1,211 – 1,344)
Morpholine	6,901 (5,042 – 9,446)
Piperidine	1,297 (1,226 – 1,370)
2-Methylpiperidine	1,032 (979 – 1,088)
4-Methylpiperidine	937 (876 – 1,002)
Hexamethyleneimine	1,163 (1,068 – 1,265)
Diisopropylamine	904 (867 – 943)
Dipropylamine	308 (291 – 325)
2-Ethylpiperidine	830 (781 – 881)
Diisobutylamine	365 (340 – 393)
Dibutylamine	313 (283 – 345)
Dipentylamine	272 (248 – 299)
Dicyclohexylamine	172 (152 – 193)

Table 10: (Continued)

Substance	**LC_{50} [µmolL^{-1}]**
Tertiary amines	
N,N-Dimethylethylamine	1,133 (1,040 – 1,235)
N,N-Diethylmethylamine	803 (714 – 903)
1-Methylpiperidine	689 (651 – 731)
N,N-Dimethylbutylamine	504 (461 – 551)
Triethylamine	598 (477 – 749)
1-Ethylpiperidine	630 (564 – 703)
N,N-Dimethylcyclohexylamine	417 (388 – 448)
N,N-Diisopropylethylamine	809 (738 – 875)
Tripropylamine	1,318 (1,165 – 1,490)
Tributylamine	1,625 (869 – 3,038)

The concentration - effect - relationship of all amines tested was steep as shown exemplary for the primary cyclohexylamine in Figure 9. This steep relationship is shown by the short distance between the approximated 0 % and 100 % mortality.

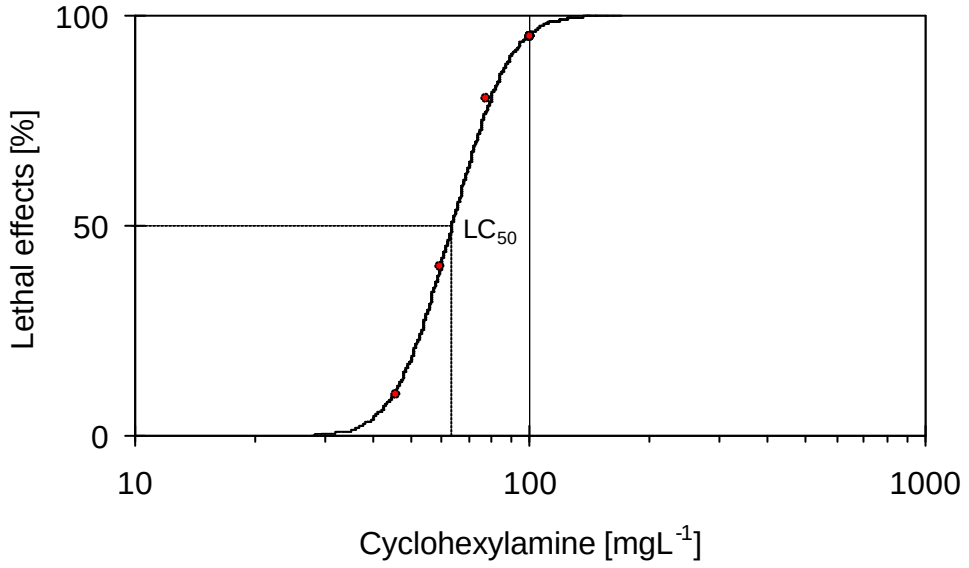


Figure 9: Concentration-effect relationship for cyclohexylamine using the *Dar*T. (Probit–Transformation; logarithmic scale; LC_{50} = 63.3 mgL^{-1} [639 µmolL^{-1}])

In general, within the series of the primary amines the toxicity of the unbranched amines increased from propylamine to decylamine (Table 10). The cyclic primary cyclohexylamine showed a toxicity which was approximately 1.5 times lower than that of the linear hexylamine. The unbranched nonylamine and decylamine were found to be the most toxic of all amines tested with 80 $\mu molL^{-1}$ and 20 $\mu molL^{-1}$, respectively. The branched isopropylamine, sec-butylamine, isobutylamine and isopentylamine were less toxic than the unbranched propylamine, butylamine and pentylamine. In general, the toxicity of the branched primary amines was approximately two times lower, compared to the unbranched amines.

Within the secondary amines the toxicity of the unbranched amines increased from diethylamine to dicyclohexylamine. Within the cyclic piperidines the highest toxicity was observed for 2-ethylpiperidine. Though it was 1.6 times more toxic compared to piperidine. The toxicity of hexamethyleneimine was in the same range of that of the piperidines. Within the group of secondary amines dicyclohexylamine was with 172 $\mu molL^{-1}$ the most toxic compound. The secondary morpholine was with 6,901 $\mu molL^{-1}$ the least toxic amine within all amines tested. The branched diisopropylamine was three times less toxic than the unbranched dipropylamine, whereas the toxicity of the branched diisobutylamine was in the same range compared to that of the unbranched dibutylamine.

In general, among the primary and secondary aliphatic amines, those with a branched chain (isopropylamine, isobutylamine, sec-butylamine, isopentylamine, diisopropylamine, diisobutyl-amine) were less toxic to the zebrafish embryos than the linear ones (propylamine, butylamine, pentylamine, dipropylamine, dibutylamine). Further, longer chains showed a higher toxicity than shorter ones. Calamari and coworkers (1980) found the same trend for seven aliphatic amines used in toxicity tests with the salmonid fish *O. mykiss*.

In contrast to this the tertiary amines showed a different toxicity pattern. The toxicity increased with increasing chain length only for molecules having not more than six carbon atoms. Amines with seven to twelve carbon atoms showed a decreasing toxicity with increasing chain length, apart from dimethylcyclohexylamine with eight carbon atoms in the molecule which was found to be the most toxic tertiary amine. Tributylamine was the least toxic amine within the tertiary amines with a calculated LC_{50} of 1,625 $\mu molL^{-1}$. Despite the use of ethanol as solubilising agent no valid experimental LC_{50} could be observed, because lethality did not exceed 15 % in the highest test concentration of 200 mgL^{-1} (Figure 10).

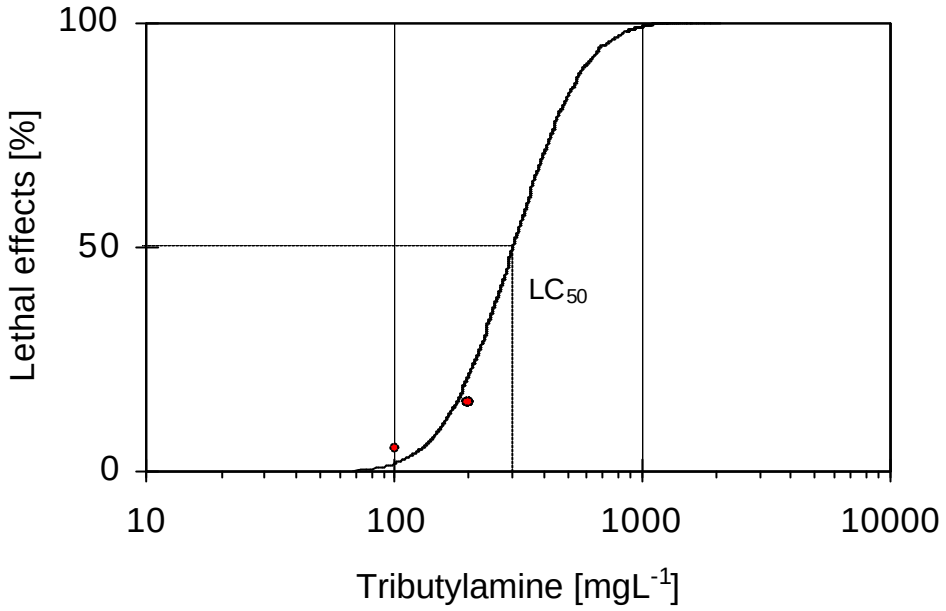


Figure 10: Concentration-effect relationship for tributylamine using the *Dar*T. (Probit–Transformation; logarithmic scale; LC_{50} = 301.2 mgL^{-1} [1,625 $\mu molL^{-1}$])

Most LC_{50} data for aliphatic amines are available for adult fish, either cyprinid or salmonid or in some cases both, and for the cladoceran *Daphnia magna*, or the green alga *Selenastrum capricornutum* (see chapter 3.1, Table 6). In most cases the data for adult fish are not suitable for a comparison with the embryo toxicity data and among themselves, because of the different test designs and species used. Further, some authors give no information about pH adjusting procedures, which make it even more difficult to interpret the differences between the fish species. Merely the common feature of test durations within the different acute fish toxicity tests can be used for a comparison among the data and thus, some trends should be discussed within this context.

For isopropylamine the LC_{50} in this study was 36 times lower than the LC_{50} (96h) of 91,200 $\mu molL^{-1}$ on the embryos of zebrafish in the investigation by Groth and coworkers (1993). However, their experiment was conducted in pH 8 adjusted test solutions, which might explain the extremely reduced toxicity, because isopropylamine was ionised to nearly 100 % at this pH.

Toxicity data of three aliphatic amines for adult *D. rerio* were available. Acute toxicity of cyclohexylamine was seven times higher in the embryotest compared with the acute toxicity found for adults (Wellens, 1982). The toxicity of diisopropylamine (Canton *et al*., 1984) and

morpholine (Wellens, 1982) in the acute fish test was approximately two times lower than in the embryotest. These data can be included in a comparison of toxicity data for adult zebrafish and embryos of zebrafish within the context of replacing the acute fish test by the embryotest (Nagel and Isberner, 1998). 44 compounds were tested using the embryotest and compared with data derived for adult zebrafish (Figure 11) and the model regression yielded:

$$\textit{log } LC_{50} \textit{ (fish)} = 0.948^{*} \textit{ log } LC_{50} \textit{ (embryo)} + 0.066 \qquad [R^2 = 0.86, \alpha=0.05]$$

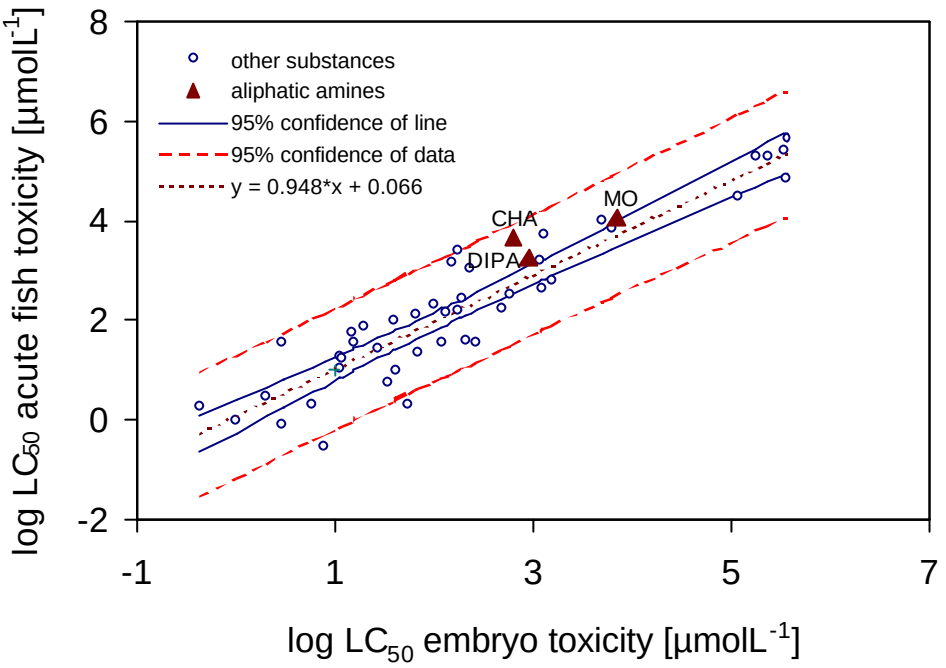


Figure 11: Correlation of LC_{50} acute fish toxicity versus LC_{50} embryo toxicity. Data of 44 substances (circles) were taken from Bachmann (1996), Maiwald (1997), and Schulte *et al.* (1996). Three data points for aliphatic amines (triangles) were added to the graph (CHA = cyclohexylamine, MO = morpholine, DIPA = diisopropyl-amine). (from Nagel and Isberner, 1998)

As to be seen in Figure 11 the three toxicity data points lay within the 95 % confidence interval of the other data. Therefore, the toxicity of these amines for adult zebrafish and the embryos of zebrafish can be described by the model regression mentioned above.

The toxicity of *Pimephales promelas* was tested using nine primary amines, and one secondary and one teriary amine, respectively. For the rainbow trout *Oncorhynchus mykiss* only five toxicity data were available. Both organisms were used in 96 h tests. The toxicity of the

primary amines on *P. promelas* increased from propylamine to decylamine. The toxicity of diethylamine with 11,690 µmolL^{-1} was the lowest compared to all amines tested. Tripropylamine was less toxic then heptylamine but more toxic than hexylamine. The toxicity of diethylamine, diisopropylamine and dibutylamine on *O. mykiss* lay in the same range. Compared to adult *D. rerio* it is obvious, that cyclohexylamine was 11 times more toxic to the rainbow trout than to the zebrafish. Diisopropylamine was approximately five times and morpholine approximately two times more toxic in the case of *O. mykiss*. Diethylamine was even 34 times more toxic to *O. mykiss* if compared to *P. promelas*. Nagel and Isberner (1998) mentioned that in general, salmonid fishes are considered to be more sensitive than cyprinid fishes. This observation can be confirmed by the comparisons performed above.

Further, a comparison of the acute fish toxicity data with the embryo toxicity data for aliphatic amines was performed (Figure 12). As can be seen the toxicity of the fathead minnow *P. promelas* can be well described by the toxicity of aliphatic amines on the embryos of zebrafish:

$$log\ LC_{50}\ (P.\ promelas) = 1.847 * log\ LC_{50}\ (D.\ rerio\ \text{embryo}) - 1.948 \quad [R^2 = 0.87, \alpha = 0.05]$$

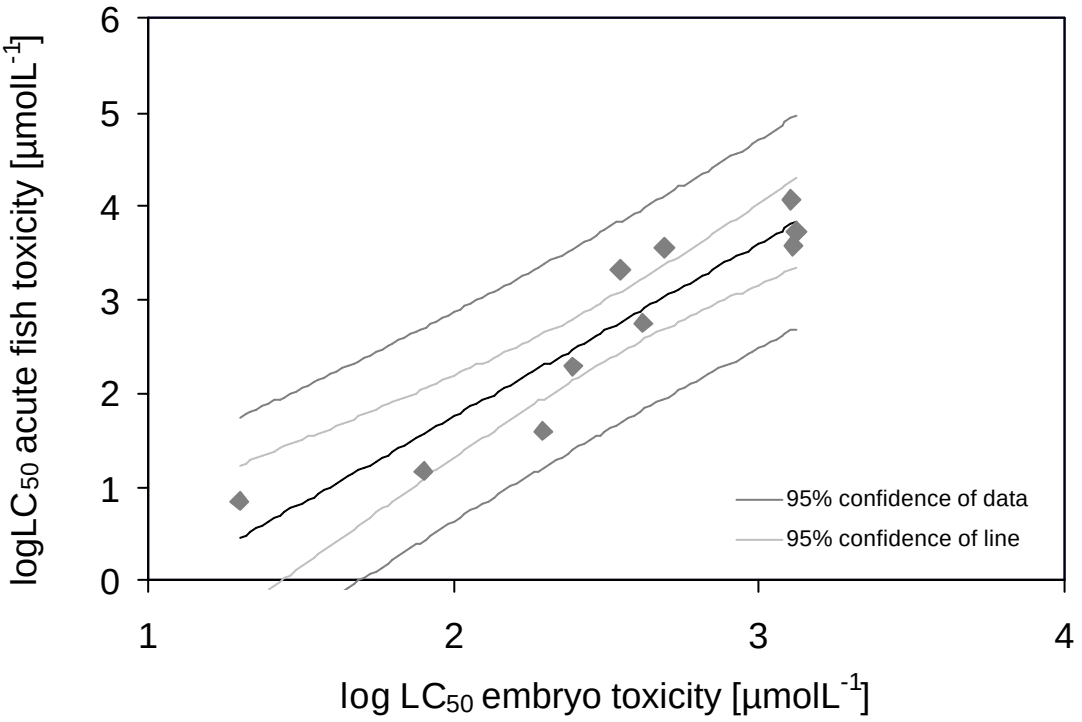


Figure 12: Comparison of LC_{50} acute fish toxicity data (96h-LC_{50} *P. promelas*) versus LC_{50} embryo toxicity for aliphatic amines (n = 10).

For *O. latipes* and *O. mykiss* not enough data were available for such a comparison.

The highest number of toxicity data were available for the creek chub *Semotilus atromaculatus*. Gillette and coworkers (1952) investigated the toxicity of 15 aliphatic amines which lead to a mortality of 100 % of the test species within 24 h. As a general, an increase of toxicity within each group of primary, secondary, and tertiary amines can be observed. In contrast to this findings a different toxicity pattern was found for the tertiary amines in the embryostests performed in this study. A comparison due to the endpoint (LC_{100}) with other toxicity data derived either from adult fish or from the embryo of zebrafish seems to be problematically and therefore, a regression with *D. rerio* embryo toxicity data was not performed.

3.2.2.3 Sublethal effects

As described by Verhaar *et al.* (1992) the aliphatic amines were assigned to narcosis type chemicals with a nonspecific mode of action and a coherency between the lipophilicity and toxicity was indicated. Nevertheless, in the study presented here some sublethal effects were found, which could indicate a specific mode of action.

Valid EC_{50}.-values could not be calculated, because the sublethal effects were covered by lethality of the embryos. In Table 11 the observed lethal effects as well as the sublethal effect “yolk sack oedema” are compiled for the primary aliphatic octylamine. At a concentration of 211.8 $\mu molL^{-1}$ 60 % lethal effects were found. This included those 55 % of the embryos which had shown no heart beat. Further, they showed sublethal effects such as yolk sack oedema. This effect could be found in only 10 % of the embryos that had survived. In the concentration of 162.5 $\mu molL^{-1}$ one coagulated embryo and two which showed yolk sack oedema could be observed. This context is illustrated exemplarly in Figure 13 for embryos exposed to octylamine.

Table 11: Lethal effects and the sublethal effect "yolk sack oedema" found in embryos exposed to octylamine.

Effect [%]	**162.5 µmolL^{-1}**	**211.3 µmolL^{-1}**	**274.7 µmolL^{-1}**
Lethal effects			
coagulation	5	5	100
no heartbeat	/	50	
Sublethal effect			
yolk sack oedema	10	70	/

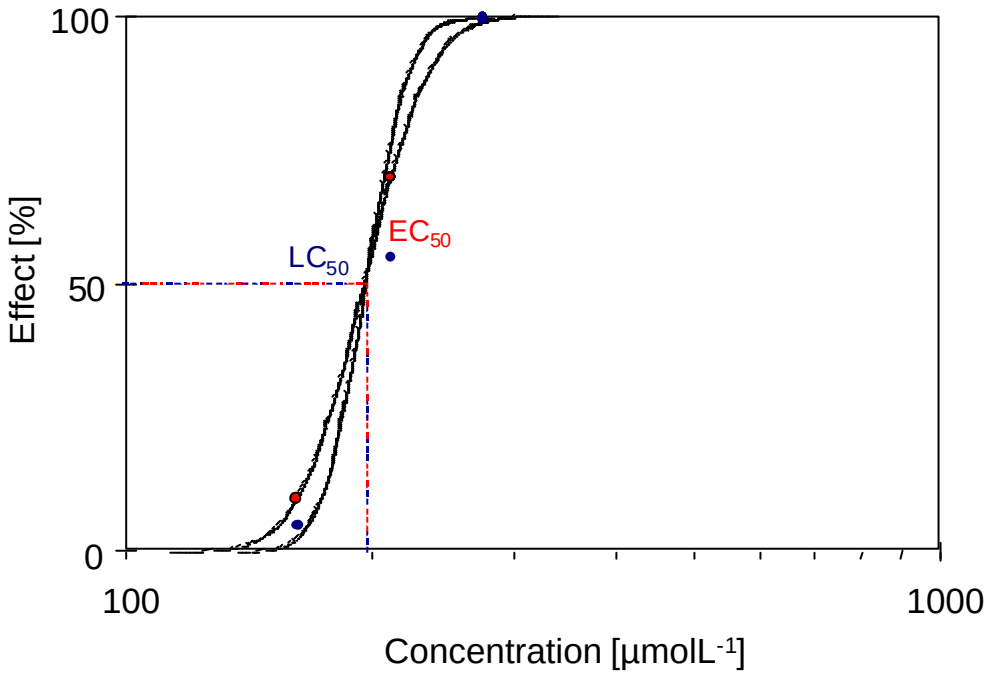


Figure 13: Concentration-effect relationship of octylamine to the embryos of zebrafish *Danio rerio* after 48h exposure. For the EC_{50} the sublethal effect "yolk sack oedema" and for the LC_{50} lethal endpoints were used (see Table 11).

Within the toxicity tests several sublethal effects could be observed, for example hypopigmentation, oedema of the yolk sack or the pericard and lack of blood circulation (Figure 14).

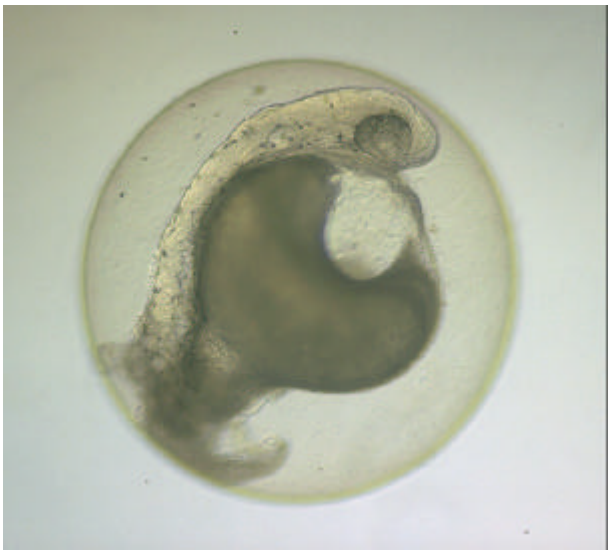


Figure 14: Effects on embryo (*Danio rerio*) after 48 h exposed to 61.5 mg/L (391 µmolL^{-1}) dipentylamine: no heart beat, no blood circulation, yolk sack oedema, deformation of tail region and hypopigmentation. (60x).

Further, missing sacculi could be observed in some cases. In those cases where the sacculi were present either otoliths (both or one) were missing (Figure 15) or granulated otoliths were observed (Figure 16).

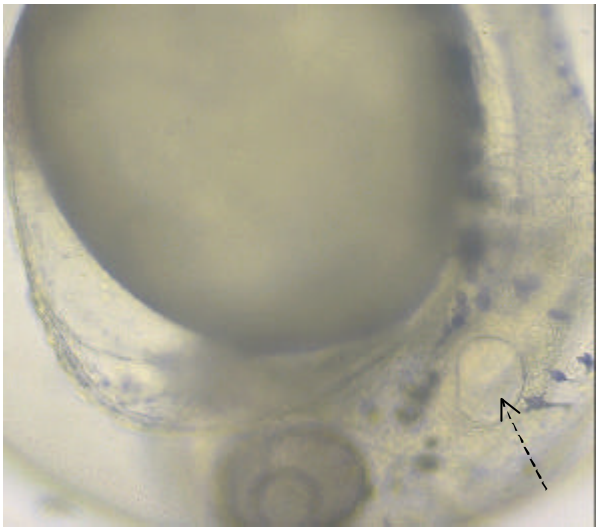


Figure 15: Sacculus of an embryo (*Danio rerio*) without otoliths after 48 h exposed to 66.7 mgL^{-1} (912 µmolL^{-1}) sec-butylamine. (100x).

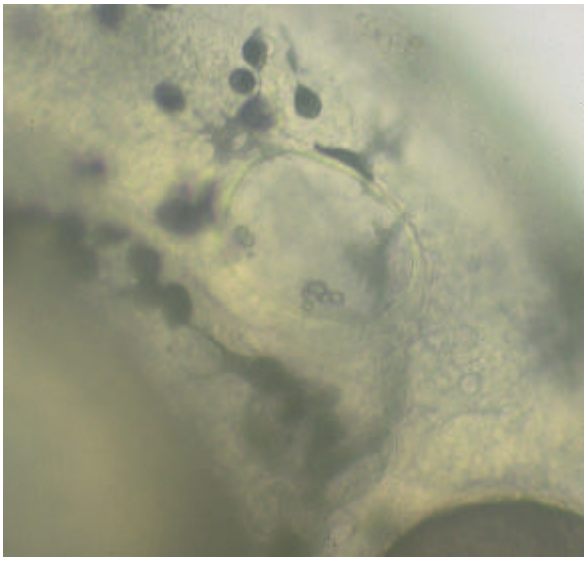


Figure 16: Granulated otoliths in the sacculus of an embryo (*Danio rerio*) after 48 h exposed to 66.7 mg/L (912 µmolL^{-1}) sec-butylamine (150x).

In very few cases a "*Spina bifida*" (Figure 17) was found in amine treatments after 24 h. These embryos died within 48 h. This phenomenon was observed in the toxicity tests performed with hexylamine, diisobutylamine, dibutylamine and dimethylbutylamine.

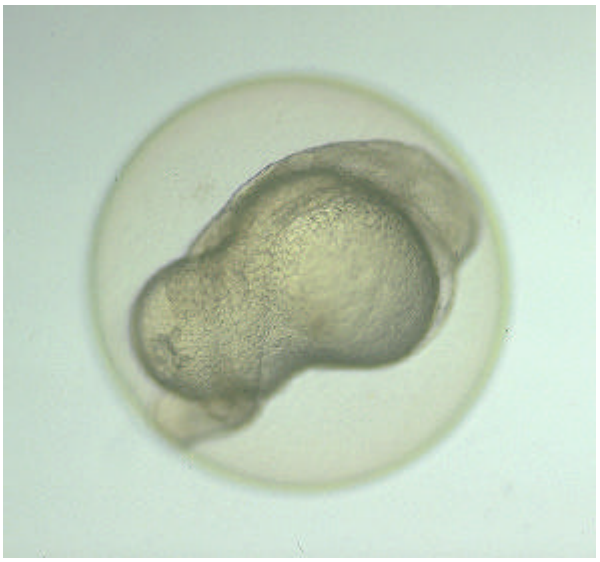


Figure 17: "*Spina bifida*" of an embryo (*Danio rerio*) after 24 h exposure to 44.4 mg/L (439 µmolL^{-1}) dimethylbutylamine (60x).

The observed "*Spina bifida*" was also described by Sander (1983). In his study he used ethanol and colcemide and proposed that this phenomenon can be caused by teratogenic

substances within the most sensitive developmental phase of ontogenesis. The first sign of aberrations can be seen in a slower epibolic movement and later in a dumb-bell shaped yolk. Embryos which showed such effects died after 24 h as observed in this study. Zeller (1995) observed this phenomenon in embryos exposed to high concentrations of propanolol, a β rezeptor blocking drug. Maiwald (1997) found this effect in embryos which were exposed to acetone, but these embryos survived until 48 h, whereas Schulte (1997) observed that embryos with a *"Spina bifida"* exposed to malathion coagulated within 24 h.

In order to examine possible patterns within or between the different groups of aliphatic amines the observed sublethal effects are summarised phenomenologically in

Table 12. Thus, only in tests with secondary and tertiary amines embryos without a sacculus could be observed, whereas this effect could not be found in tests with primary amines. Granulated otoliths were found in primary as well as secondary amines. For tertiary amines this effect could be observed only for 1-ethylpiperidine, triethylamine and tripropylamine. Embryos which had only one otolith were observed in primary and secondary amines occasionally, whereas this effect was more common in tertiary amines. The phenomenon "no otoliths" was found for embryos which were exposed to primary, secondary as well as to tertiary amines.

Oedema of the pericard could be observed occasionally in all groups of tested amines. Schulte (1997) and Maiwald (1997) observed this effect in embryos which were exposed to lindane.

Hypopigmentation was observed in all tests with amines, except for cyclohexylamine and dimethylbutylamine. Yolk sack oedema were found in all tests. This effect is usually combined with lethal effects. Schulte (1997) found for several anilines and phenols that the embryos showed both a reduced pigmentation and yolk sack oedema. For embryos exposed to *p-tert*-butylphenol a concentration dependent hypopigmentation could be observed (Maiwald, 1997).

Further, it was mentioned in the introduction that cyclohexylamine is suspected as having a teratogenic potential (Klaasen *et al*., 1986). No teratogenic effects could be observed in the embryos of zebrafish.

Table 12: Compilation of the sublethal effects of aliphatic amines on the embryos of *Danio rerio* after 48 h exposure.

Substance	**Effects**							
	"*Spina bifida*"	no sacculus	granulated otoliths	one otolith	no otoliths	hypopigmentation	yolk sack oedema	pericard oedema
Primary amines								
n-Propylamine					•	•	•	•
Isopropylamine					•	•	•	•
n-Butylamine			•			•	•	•
sec-Butylamine			•	•	•	•	•	
Isobutylamine					•	•	•	
n-Pentylamine			•			•	•	•
Isopentylamine			•	•		•	•	
Cyclohexylamine					•		•	
n-Hexylamine	•					•	•	
n-Heptylamine			•		•	•	•	
n-Octylamine			•			•	•	•
n-Nonylamine			•		•	•	•	•
n-Decylamine			•			•	•	

Table 12: (Continued)

Substance	**Effects**							
	"*Spina bifida*"	no sacculus	granulated otoliths	one otolith	no otoliths	hypopigmentation	yolk sack oedema	pericard oedema
Secondary amines								
Diethylamine		•	•		•	•	•	•
Morpholine		•	•	•		•		•
Piperidine		•	•	•		•		•
2-Methylpiperidine		•			•	•	•	
4-Methylpiperidine		•			•	•	•	
Hexamethyleneimine		•				•	•	•
Diisopropylamine		•			•	•	•	•
Dipropylamine		•	•	•		•	•	•
2-Ethylpiperidine		•	•	•	•	•	•	
Diisobutylamine	•		•			•	•	
Dibutylamine	•		•	•		•	•	
Dipentylamine			•			•	•	•
Dicyclohexylamine		•	•			•	•	

Table 12: (Continued)

Substance	**Effects**							
	"*Spina bifida*"	no sacculus	granulated otoliths	one otolith	no otoliths	hypopigmentation	yolk sack oedema	pericard oedema
Tertiary amines								
N,N-Dimethyl-ethylamine		•		•	•	•	•	•
N,N-Diethyl-methylamine		•		•	•	•	•	
1-Methylpiperidine		•			•	•	•	
N,N-Dimethyl-butylamine	•	•		•			•	
Triethylamine		•	•	•	•	•	•	•
1-Ethylpiperidine			•		•	•	•	
N,N-Dimethylcyclo-hexylamine		•		•	•	•	•	
N,N-Diisopropyl-ethylamine		•				•	•	
Tripropylamine		•	•			•	•	•
Tributylamine				•		•	•	•

3.3 Bioconcentration of aliphatic amines in the embryos of *Danio rerio*

3.3.1 Uptake of ^{14}C-butylamine

In aquatic ecotoxicology the toxicity of pollutants are expressed as EC_x-values which refer to the concentration of a chemical in the waterphase. But as a rule, the dose of a chemical which affecting the organisms is more meaningful. ED_x-values enable the comparison of effects between chemicals and/or organisms on the basis of body-related doses. Moreover, within this approach intrinsic properties of compounds are becoming more relevance.

To predict the lethal dose of aliphatic amines to the embryos of *Danio rerio* the model compound ^{14}C-butylamine was used. Therefore, in a static test the bioconcentration of ^{14}C-butylamine over the time was measured. The equation 2 (see chapter 2.4) was fitted to experimental data. The uptake of ^{14}C-butylamine in the eggs can be successfully described by a one-compartment first-order-kinetics model. (Figure 18).

$$y = 4.142*(1-exp^{-0.279*x})$$

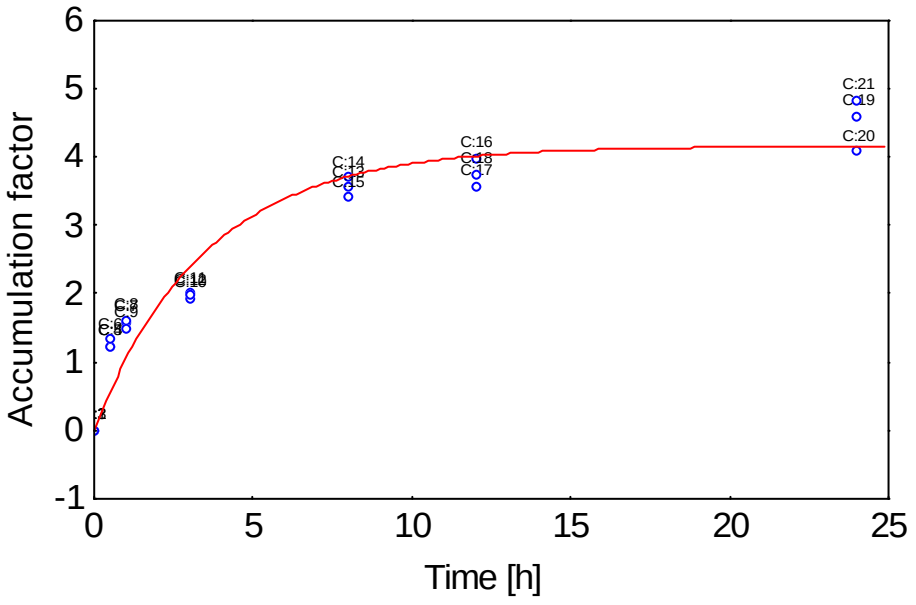


Figure 18: Accumulation of ^{14}C-butylamine in the eggs of *Danio rerio* [R^2 = 0.957] within an exposure time 24 h. (c_x = three samples of n = 7 eggs per sampling point were taken for counting radioactivity)

In the experiment a steady state between uptake and elimination of ^{14}C-butylamine occurred within approximately 10 h. At the end of exposure samples of toluene and KOH were taken (1mL each) and the activity was counted. After 24 h 1.4 % of the total ^{14}C-butylamine activity were detected in toluene. In the bottle with KOH only 0.1% of the total activity was found at the end of exposure. This activity corresponds to $^{14}CO_2$ which is an indication for mineralisation during the experiment. These values indicate, that the amount of evaporated butylamine can be neglected and further that the production of carbondioxide was very low during the exposure.

Recently some experiments to determine the uptake of chemicals in the eggs of zebrafish have been performed (Ensenbach, 1987; Ensenbach, 2000, pers. comm.). In Table 13 the lipophilicity (log K_{ow}) and the determined bioconcentration factors (BCF) of the tested compounds are presented.

Table 13: Bioconcentration factors (BCF and logBCF) and lipophilicity (log K_{ow}) of several compounds in the eggs of *Danio rerio*.

Substance	**log K_{ow}**	**BCF [egg]**	**log BCF [egg]**
Methanol[a]	-0.72	0.48	-0.32
Phenol[a]	1.48	4	0.6
4-Nitrophenol[a]	1.9	4.9	0.69
3,4-Dichloroaniline[a]	2.42	33	1.52
4- Chlorophenol[a]	2.44	23.6	1.37
Pentachlorophenol[a]	3.65	37.2	1.57
Lindane[a]	3.72	110	2.04
Endosulfane[a]	4.65	108	2.03
Butylamine[b]	0.97	4.14	0.64

[a] data taken from Ensenbach (1987) and Ensenbach (2000, pers.comm.)
[b] investigated in this study

The determined BCF´s can be used to predict the relationship between the bioconcentration and the lipophilicity (Figure 19). The relationship can be described with a linear regression model:

$$log\ BCF = 0.461 * log\ K_{ow} + 0.0769 \qquad [n = 9;\ R^2 = 0.92;\ r = 0.96]$$

The Figure 19 shows a good coherency between the bioconcentration and the lipophilicity: the more lipophilic a substance the higher its accumulation in an organism. This relationship does not exist if the log K_{ow} > 6 (Könemann and van Leeuwen, 1980; Nendza, 1991). Though, in this case the statement can be neglected as endosulfane was the compound with the highest lipophilicity of nearly five.

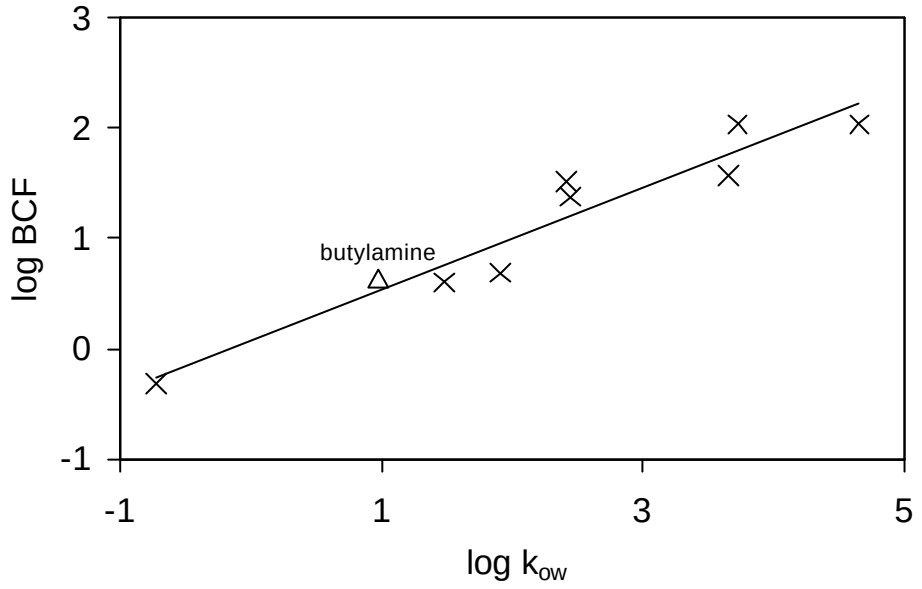


Figure 19: Bioconcentration of several compounds in the eggs of *Danio rerio* (Ensenbach, 1987; Ensenbach, 2000, pers. comm.) and of ^{14}C-butylamine determined in this study.

Unfortunately, it was not possible to test whether this relationship is also valid for amines with higher lipophilicity. For this reason, uncertainty concerning the use of this regression remains.

3.4 Calculation of lethal doses of aliphatic amines for the embryos of *Danio rerio*

The calculated bioconcentration factors based on experimental results and the observed LC_{50}´s (see chapter 3.2.2.2) can be used to predict lethal doses (LD_{50}^{*}) of aliphatic amines for the embryos of zebrafish using the following equation 7:

(7) $LD_{50} = LC_{50} * BCF$ or $\log LD_{50} = \log LC_{50} + \log BCF$

In Table 14 the LC_{50}-values, the calculated BCF´s and the resulting LD_{50}^{*}´s of aliphatic amines for the embryos of *Danio rerio* are presented.

Table 14: Compilation of LC_{50}-values, calculated bioconcentration factors (BCF) and calculated LD_{50}^{*}-values of aliphatic amines for the embryos of *Danio rerio.*

Substance	**LC_{50} [µmolL^{-1}]**	**BCF**	**LD_{50}^{*} [µmolkg^{-1}]**
Primary amines			
n-Propylamine	1,339	1.99	2,664
Isopropylamine	2,531	1.57	3,973
n-Butylamine	491	3.34	1,640
sec-Butylamine	1,301	2.62	3,409
Isobutylamine	1,267	2.59	3,282
n-Pentylamine	354	5.80	2,053
Isopentylamine	678	5.39	3,654
Cyclohexylamine	639	5.80	3,706
n-Hexylamine	418	10.63	4,443
n-Heptylamine	247	18.27	4,513
n-Octylamine	197	25.93	5,108
n-Nonylamine	80	43.62	3,490
n-Decylamine	20	73.38	1,468

Table 14: (Continued)

Substance	**LC_{50} [µmolL^{-1}]**	**BCF**	**LD_{50}^{*} [µmolkg^{-1}]**
Secondary amines			
Diethylamine	1,275	2.21	2,818
Morpholine	6,901	0.48	3,312
Piperidine	1,297	2.91	3,774
2-Methylpiperidine	1,032	4.55	4,696
4-Methylpiperidine	937	4.55	4,263
Hexamethyleneimine	1,163	7.10	8,257
2-Ethylpiperidine	830	7.65	6,350
Diisopropylamine	904	5.28	4,773
Dipropylamine	308	7.03	2,165
Diisobutylamine	365	20.53	7,493
Dibutylamine	313	24.07	7,534
Dipentylamine	272	68.13	18,531
Dicyclohexylamine	172	106.4	18,301
Tertiary amines			
N,N-Dimethylethylamine	1,133	2.51	2,844
N,N-Diethylmethylamine	803	4.22	3,389
1-Methylpiperidine	689	4.74	3,266
N,N-Dimethylbutylamine	504	7.25	3,654
Triethylamine	598	5.56	3,325
1-Ethylpiperidine	630	7.65	4,820
N,N-Dimethylcyclohexylamine	417	11.95	4,983
N,N-Diisopropylethylamine	809	17.69	14,311
Tripropylamine	1,318	23.07	30,406
Tributylamine	1,625	109.8	178,425

3.5 QSAR modelling

3.5.1 Structure-Toxicity Relationships

To investigate the structure-toxicity relationship first all aliphatic amines were examined using the median lethal concentration (LC_{50}) and the adjusted lipophilicity (see chapter 2.5) as descriptor. The estimated log K_{ow} values were adjusted to known experimental log K_{ow} values to make them better comparable with the experimentally derived toxicity data. In the following the adjusted lipophilicity is given as the log K_{ow}.

Figure 20 shows that there is no clear dependence of the toxicity LC_{50} on the lipophilicity. With 0.48 the regression coefficient (R^2) was very low. Using the "leave several out" (LSO) method the crossvalidation coefficient (Q^2) was only 0.35 and showed that there is no relationship between both parameters. However, it is obvious that in most cases the primary amines are more toxic than the secondary and tertiary amines at similar values of log K_{ow}. This is in good agreement with investigations by Veith and Mekenyan (1993). They mentioned that the toxicity of type II chemicals or polar narcotics increases unlike that of the type I or non-polar acting narcotics. As aforementioned in the introduction the primary amines are defined as polar and the secondary and tertiary amines as non-polar acting chemicals.

Because of the non-significant relationship between lipophilicity and toxicity for all aliphatic amines, the primary amines were examined separately (Figure 21). For this subclass of compounds a clear coherency can be seen. The toxicity increased with increasing lipophilicity and can be described using a simple linear regression model:

$$log(1/LC_{50}) = 0.467 * log\ K_{ow} - 3.429 \qquad (n = 13;\ R^2 = 0.91;\ Q^2 = 0.85\ [LOO]).$$

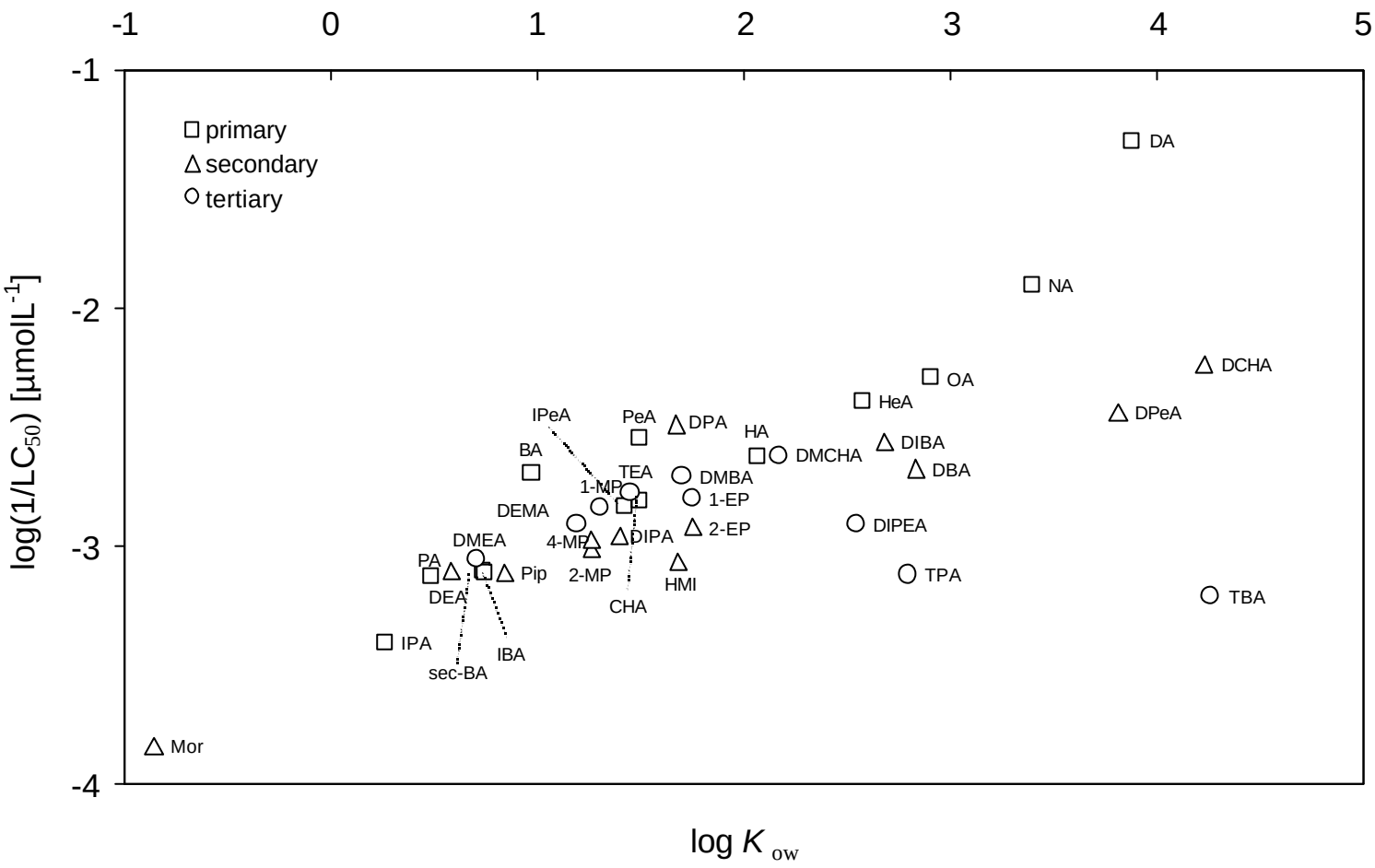


Figure 20: Relationship between toxicity (LC_{50}) and lipophilicity (log K_{ow}) of aliphatic amines.

(A = amine; PA propyl-; IPA isopropyl-, BA butyl-, IBA isobutyl-, sec-BA sec-butyl-, PeA isopentyl-, IpeA isopentyl-, CHA cyclohexyl-, HA hexyl-, HeA heptyl-, NA nonyl-, DA decyl-; DEA ditehyl-, DPA dipropyl-, DIPA diisopropyl-, DBA dibutyl-, DIBA diisobutyl-,DPeA dipentyl-, Pip piperidine, Mor morpholine, 2-MP 2-methylpiperidine, 4-MP 4-methylpiperidine, 2-EP 2-ethylpiperidine, HMI hexamethyleneimine, DCHA dicyclohexylamine; TEA triethyl-, TPA tripropyl-, TBA tributyl-, DMCHA dimethylcyclohexyl-, DMEA dimethylethyl-, DEMA diethylmethyl-, DMBA dimethylbutyl-, DIPEA diisopropylethyl-, 1-MP 1-methylpiperidine, 1-EP 1-ethylpiperidine)

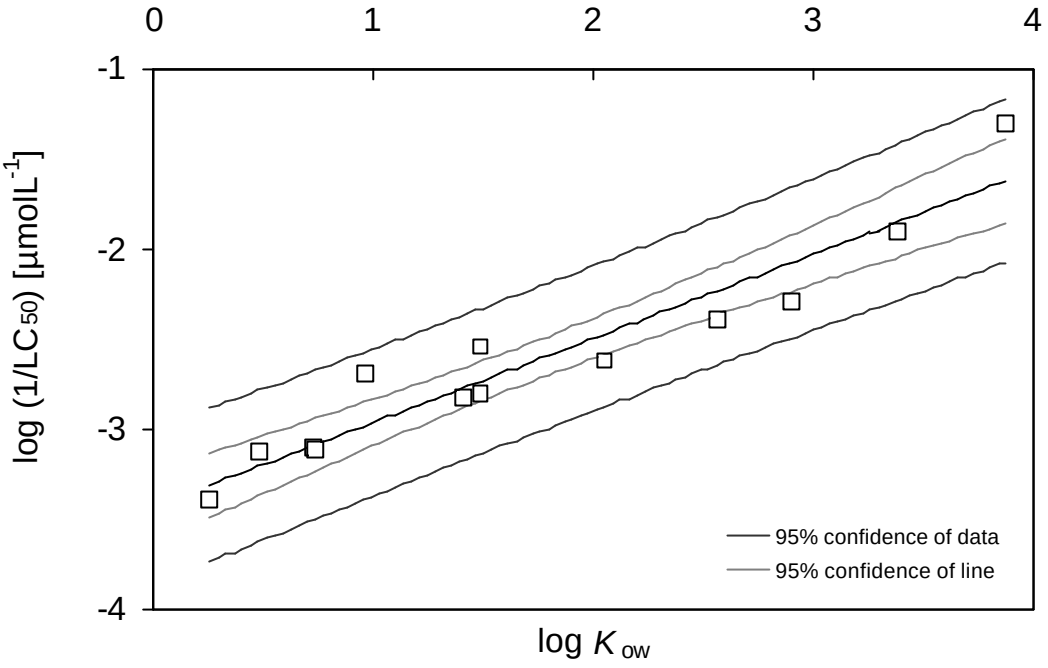


Figure 21: Relationship between toxicity (LC_{50}) and lipophilicity (log K_{ow}) of primary aliphatic amines

Also for the secondary amines a linear regression model could be found (Figure 22):

$$log(1/LC_{50}) = 0.278*log\ K_{ow} - 3.370 \qquad (n = 13;\ R^2 = 0.85;\ Q^2 = 0.77\ [LOO]).$$

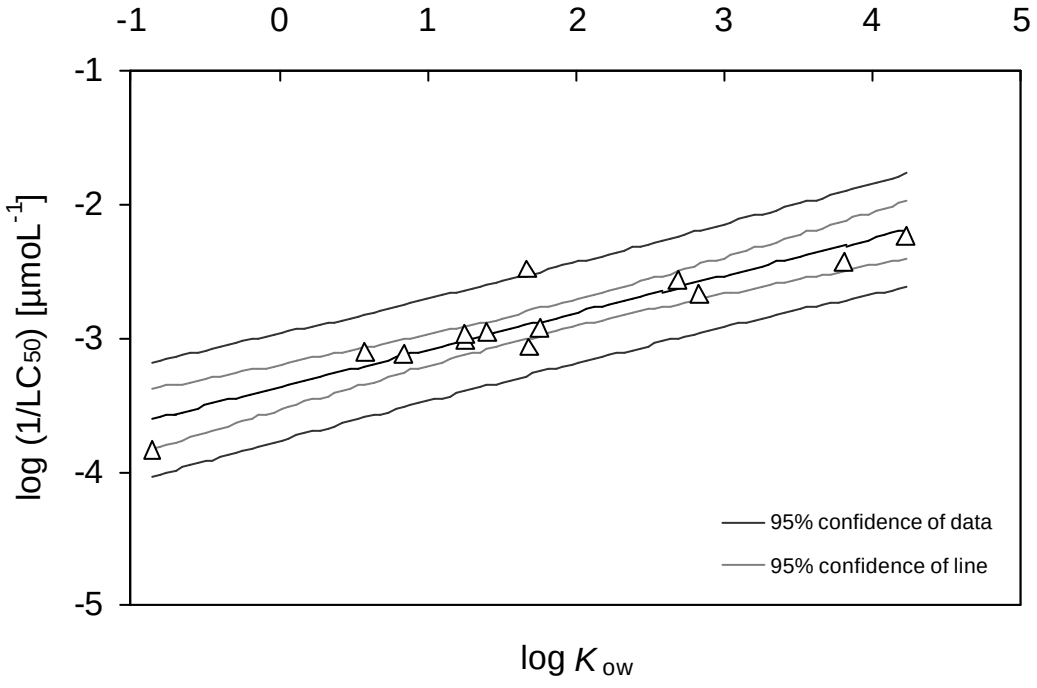


Figure 22: Relationship between toxicity (LC_{50}) and lipophilicity (log K_{ow}) of secondary aliphatic amines

Both regressions showed that the relationship between toxicity and lipophilicity of the primary and secondary aliphatic amines is significant. However, the slope of the regression model for the secondary amines differs from that of the model for the primary amines by approximately a factor of two (regressions based on Figure 22 and Figure 23). This finding is in contrast to a statement given by Cronin and Dearden (1995). They mentioned that despite a significant relationship between lipophilicity and toxicity in the case of polar narcotics a lower slope and a higher intercept in comparison to non-polar narcotics could be found. However, the basis for the relationships of the non-polar compounds were a 14 day LC_{50} performed with the guppy *Poecilia reticulata* (Könemann, 1981) and for the polar compounds performed with the fathead minnow *Pimephales promelas* (Veith and Broderius, 1987).

In the case of tertiary amines the toxicity increased with increasing lipophilicity up to a log K_{ow} of approximately 2 and then the toxicity of higher lipophilic tertiary amines decreased. For a simple linear regression of toxicity versus lipophilicity only a regression coefficient of R^2 = 0.006 could be found. Therefore, a bilinear model seems to be more suitable to describe the relationship between both parameters (Figure 23). The bilinear equation of logK_{ow} which was fitted to the measured data is:

$$log(1/LC_{50}) = 0.424*log\ K_{ow} - 5.945*\log(0.00073*K_{ow} + 1) - 3.356$$

The regression coefficient for this model was only R^2 = 0.43 with an extremely negative Q^2 of -86. This is not surprising, because for tributylamine a difference of approximately 5 between the observed and the calculated toxicity was found (Figure 23). Therefore, tributylamine was omitted from the regression analysis and the resulting R^2 and Q^2 were then 0.915 and 0.684, respectively.

Tributylamine might be defined as an outlier within the data set of tertiary amines, because as aforementioned the LC_{50} of this compound was not determined properly. On the other hand, a nearly 99 % ionisation was measured in the test solutions (t_0). Therefore, only a neglectible amount of non-dissociated molecules was in the water at the beginning of exposure. Thus, the "real" toxicity could be higher than observed.

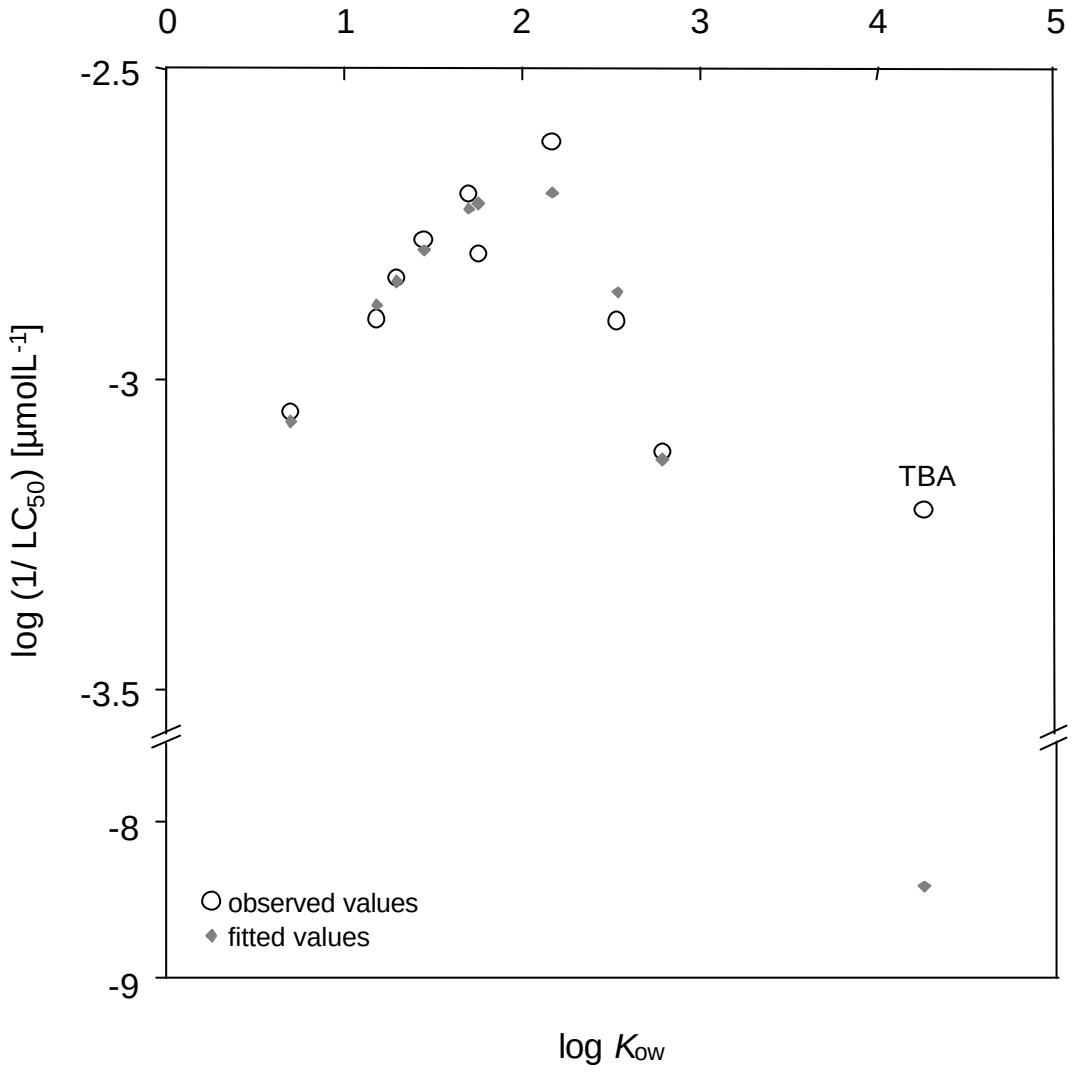


Figure 23: Relationship between toxicity (LC_{50}) and lipophilicity (log K_{ow}) of tertiary aliphatic amines (TBA = tributylamine)

However, there is no mechanistic background for this bilinear function and the dataset of nine compounds is very small. Further, there is a gap of data for the tertiary amines between a log K_{ow} of 3 to 4 (Figure 23), and thus two or three tertiary amines within this range should be tested using the embryotest in order to accept or reject the derived bilinear model.

In addition to the lipophilicity also the steric properties of the molecules are of interest, because the molecules have to pass the chorion, the perivitellin space and the embryo membranes. Several QSAR studies showed that the effect concentrations are predominantly correlated with the log K_{ow} (Schultz *et al.*, 1991b) and with electronic descriptors like the pK_a, log *D* as distribution coefficient parameter or $^1\chi^v$ as the first order valence corrected molecular

connectivity index (Newsome *et al.*, 1991). Protic and Sabljic (1989) compared the toxicity of chemicals to the fathead minnow using the valence zero-order molecular connectivity index ($^0\chi^v$). In their study they found a significant correlation for nine aliphatic amines including two diamines and seven monoalkylamines. In contrast, no good relationship between the toxicity and the molecular connectivity indices could be found for the 36 aliphatic amines discussed here (Table 15).

Table 15: Intercorrelation between toxicity and several molecular connectivity indices of aliphatic amines [log ($1/LC_{50}$) in $\mu molL^{-1}$].

	$^0\chi$	$^1\chi$	$^2\chi$	$^3\chi^p$	$^3\chi^c$	$^0\chi^v$	$^1\chi^v$	$^2\chi^v$	$^3\chi^{p,v}$
log ($1/LC_{50}$)	0.54	0.53	0.38	0.35	-0.20	0.52	0.55	0.47	0.44

The degree of ionisation as modelled by pK_a provided no additional improvement of the results. Thus, the toxicity of amines can not be described by the pK_a of these compounds, as shown in Figure 24. This may reflect the fact that most of the compounds show little variation in pK_a. The same tendency was found by Newsome and coworkers (1991). They indicated that there is no coherency for 41 amines studied.

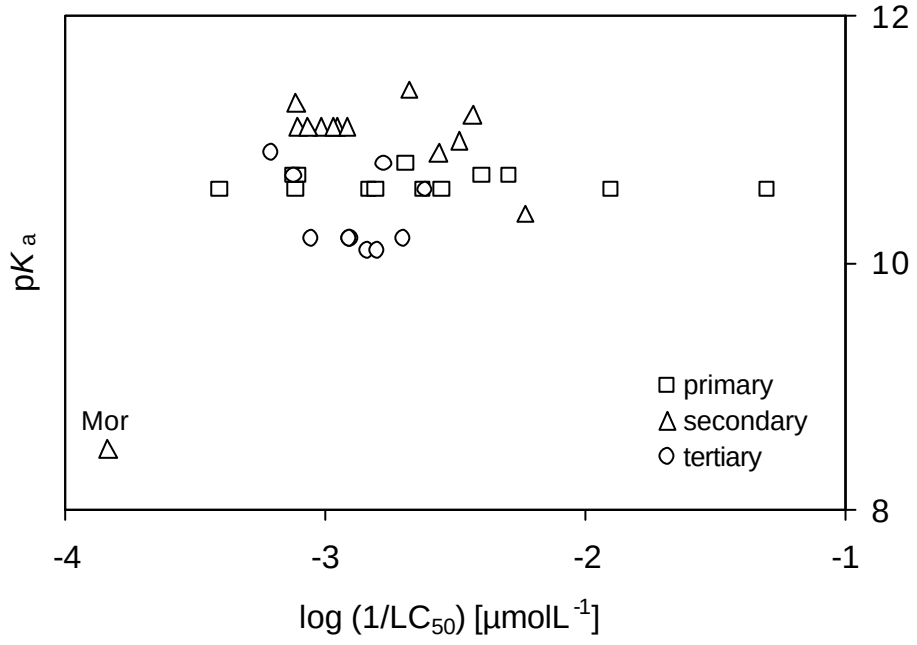


Figure 24: Relationship between the toxicity and pK_a of 36 aliphatic amines ($R^2 = 0.054$, if morpholine included; $R^2 = 0.012$, if morpholine omitted from regression).(Mor = morpholine)

Using the intercorrelation matrix it was found that for the aliphatic amines most connectivity indices, such as $^{1}\chi^{v}$, are well correlated with the log K_{ow}, and are therefore not suitable for a multiple regression (Table 16).

Table 16: Intercorrelation between lipophilicity (log K_{ow}) and several molecular connectivity indices of aliphatic amines.

	$^{0}\chi$	$^{1}\chi$	$^{2}\chi$	$^{3}\chi^{p}$	$^{3}\chi^{c}$	$^{0}\chi^{v}$	$^{1}\chi^{v}$	$^{2}\chi^{v}$	$^{3}\chi^{p,v}$
log K_{ow}	0.96	0.95	0.85	0.77	-0.04	0.96	0.97	0.89	0.81

Using multiple regression technique the best fit for describing the toxicity of all aliphatic amines tested was found with the adjusted lipophilicity (log K_{ow}), the effective diameter (D_{eff}) and the maximum positive charge on hydrogen atom (H^{+}_{max}):

$$log(1/LC_{50}) = 0.343*log\ K_{ow} - 0.269*D_{eff} - 1.73*H^{+}_{max} - 1.342$$

(n = 36; R^2 = 0.824; Q^2 = 0.763 [LSO]; F = 55.54; P < 0.0001).

There is no good correlation between log K_{ow} and D_{eff} (r = 0.32), and between the log K_{ow} and H^{+}_{max} (r = - 0.07) using the intercorrelation coefficients (Appendix, Table A2). Therefore, these descriptors were suitable for the multiple regression model including all homologous aliphatic amines. Further, as shown in Figure 20 the R^2 was 0.48 for the simple relationship between toxicity and lipophilicity. Thus, the highly significant relationship indicates that the toxicity of all aliphatic amines can be best described if the three descriptors are included in a threefactorial regression model.

The toxicity of the subclass of primary amines can be described as:

$$log(1/LC_{50}) = 0.439*log\ K_{ow} - 0.157*D_{eff} - 2.479$$

(n = 13; R^2 = 0.915; Q^2 = 0.866 [LOO]; F = 65.59; P < 0.0001).

The toxicity of of the subclass secondary amines can be described as:

$$log(1/LC_{50}) = 0.293*log\ K_{ow} - 0.107*D_{eff} - 2.711$$

(n = 13; R^2 = 0.851; Q^2 = 0.714 [LOO]; F = 35.22; P < 0.0001).

These two models show that no additional improvement will be gained if the effective diameter as descriptor is included. As shown in Figure 21 and Figure 22 the lipophilicity alone is adequate to describe the toxicity of primary ($R^2 = 0.91$) and secondary amines ($R^2 = 0.85$).

Using several molecular descriptors (physicochemical, geometrical, quantum-chemical and topological) no multiple regressions with $R^2 > 0.5$ could be found for the tertiary amines. For this group a dependence on the diameter of molecules was assumed, which may be the reason for an inhibition of membrane permability resulting in a lower toxicity with increasing lipophilicity. The assumption might be confirmed by the multiple regression model calculated for all aliphatic amines. Further, Opperhuizen *et al.* (1985) reported a loss in membrane permeability with molecules having widths greater then 0.95 nm. The effective diameter of tripropylamine and tributylamine is 0.986 nm and 1.079 nm, respectively. Therefore, an inhibition might be expected. In their study about the 96-h fathead minnow toxicity Newsome and coworkers (1991) established that for the tertiary amines - when examined as subclass - no satisfactory correlation with the lipophilicity could be found. Moreover, they suggest that steric effects might be inadequately parameterised.

Using the mathematical model developed for all tested aliphatic amines predicted LC_{50}´s can be calculated and compared with the experimentally derived LC_{50} (Figure 25).

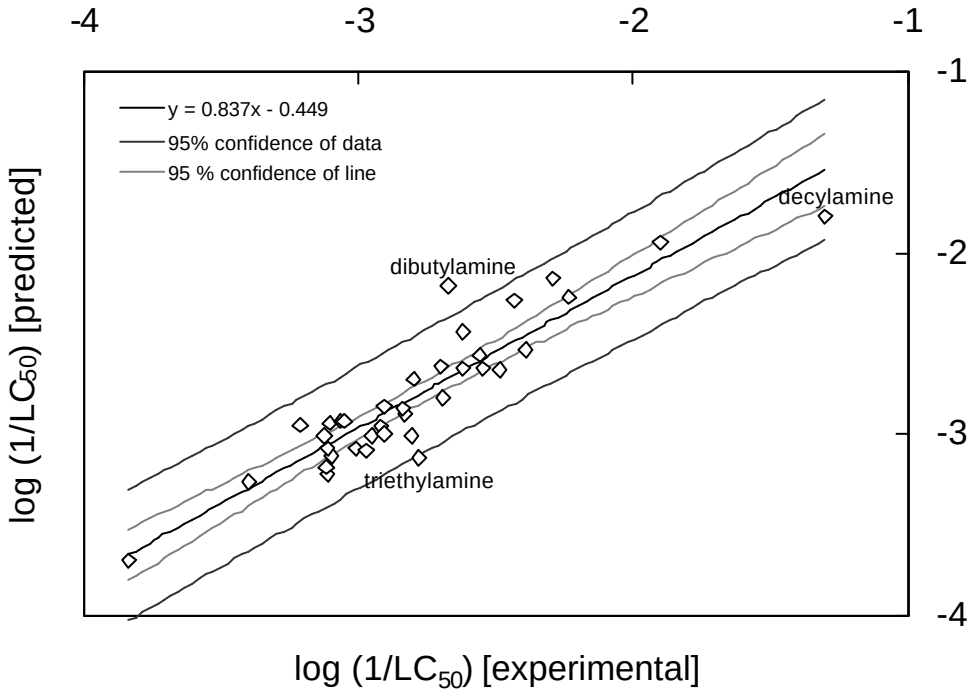


Figure 25: Calculated versus experimental LC_{50} of aliphatic amines for the embryos of *Danio rerio* ($n = 36$; $R^2 = 0.838$; $r = 0.916$)

The relationship between the experimental and calculated data is strong and dibutylamine and triethylamine are the only outside of the 95% confidence band for the data. A simple explanation is not possible, the misfitting may be due to unreliable experimental data. The predicted LC_{50} of dibutylamine is lower than the experimental LC_{50}, whereas the predicted LC_{50} of triethylamine is greater than the experimentally derived LC_{50}. Lipnick (1991) indicated that the degree to which a compound exists as an outlier with respect to the baseline narcosis model is reflected in the so called calculated excess toxicity (T_e). This baseline narcosis model is based on the relationship between toxicity and lipophilicity. However, as aforementioned the aliphatic amines studied here can be described best by a three parameter multiple regression model. Nevertheless, the excess toxicity should be calculated based on the multiple regression using equation 8:

(8) $$T_e = \frac{LC_{50(predicted)}}{LC_{50(observed)}}$$

where T_e is the ratio between predicted and observed toxicity (Table 17).

Table 17: Excess toxicity (T_e) for aliphatic amines. Highlighted values distinguish a strongly under- or overestimated toxicity (<0.5; >2.0)

Substance	**$LC_{50\ (observed)}$ [a]**	**$LC_{50\ (predicted)}$ [b]**	**T_e**
Primary amines			
n-Propylamine	1,339	1,019	0.8
Isopropylamine	2,531	1,812	0.7
n-Butylamine	491	633	1.3
sec-Butylamine	1,301	1,203	0.9
Isobutylamine	1,267	1,337	1.1
n-Pentylamine	354	431	1.2
Isopentylamine	678	774	1.1
Cyclohexylamine	639	1,034	1.6
n-Hexylamine	418	273	0.7
n-Heptylamine	247	346	1.4
n-Octylamine	197	137	0.7
n-Nonylamine	80	88	1.1
n-Decylamine	20	63	**3.2**

Table 17: (Continued)

Substance	**$LC_{50\ (observed)}$** [a]	**$LC_{50\ (predicted)}$** [b]	**T_e**
Secondary amines			
Diethylamine	1,275	872	0.7
Morpholine	6,901	4,968	0.7
Piperidine	1,297	1,675	1.3
2-Methylpiperidine	1,032	1,191	1.2
4-Methylpiperidine	937	1,237	1.3
Hexamethyleneimine	1,163	858	0.7
Diisopropylamine	904	1,029	1.1
Dipropylamine	308	446	1.4
2-Ethylpiperidine	830	902	1.1
Diisobutylamine	365	368	1.0
Dibutylamine	473	151	**0.3**
Dipentylamine	272	181	0.7
Dicyclohexylamine	172	173	1.0
Tertiary amines			
N,N-Dimethylethylamine	1,133	857	0.8
N,N-Diethylmethylamine	803	1,005	1.3
1-Methylpiperidine	689	714	1.0
N,N-Dimethylbutylamine	504	424	0.8
Triethylamine	598	1,366	**2.3**
1-Ethylpiperidine	630	502	0.8
N,N-Dimethylcyclohexylamine	417	431	1.0
N,N-Diisopropylethylamine	809	700	0.9
Tripropylamine	1,318	1,513	1.1
Tributylamine	1,625	898	0.6

[a] observed toxicity using the embryotest with *Danio rerio*

[b] predicted LC_{50} based on model equation: $log(1/LC_{50}) = 0.343 * log\ K_{ow} - 0.269 * D_{eff} - 1.73 * H^{+}_{max} - 1.342$; (log K_{ow}-adjusted)

The predicted toxicity of decylamine is three times lower as compared to the observed toxicity. However, as to be seen in Figure 25 the relationship for both experimental and calcluated toxicity lay within the 95 % confidence of data, but outside of the 95 % of line, and is therefore not defined as an outlier of this data set. For dibutylamine and triethylamine a three times lower and a two times higher toxicity was predicted by the corresponding equation, respectively. There is no explanation for the differences between observed and calculated toxicity.

As mentioned in the introduction of this study primary amines are defined as acting by polar and the secondary as well as the tertiary amines by non-polar narcosis within a QSAR modelling of the fathead minnow acute toxicity database (Nendza and Russom, 1991; Verhaar *et al*., 1992). In general, the toxicity of narcotic acting chemicals can be described best by their lipophilicity and a linear relationship is given. But for the case of the tertiary amines the relationship between toxicity and lipophilicity could be described by a bilinear regression model. Therefore, the tertiary amines should be better exclude from further discussions about non-polar narcosis.

The subclass of secondary aliphatic amines and in addition toxicity data of three alcohols, 1-octanol (Schulte, 1997), ethanol and aceton (Maiwald, 1997), can be investigated as a group defined as non-polar acting narcotics. Könemann (1981) suggested a strong relationship between the toxicity and the lipophilicity for 50 non-polar acting compounds tested within the 14 d *Poecilia reticulata* assay. In contrast, for the secondary amines and the alcohols and acetone, no significant relationship between this two parameters could be found. The regression coefficient was $R^2 = 0.643$. Ethanol and acetone were clearly identified as outliers (Figure 26). This finding is difficult to explain as the log K_{ow} of morpholine is lower than that of ethanole and acetone, but its toxicity was higher. While regression was improved to $R^2 = 0.78$, if these both were omitted from the dataset, the linear regression model for the secondary aliphatic amines alone was better ($R^2 = 0.85$).

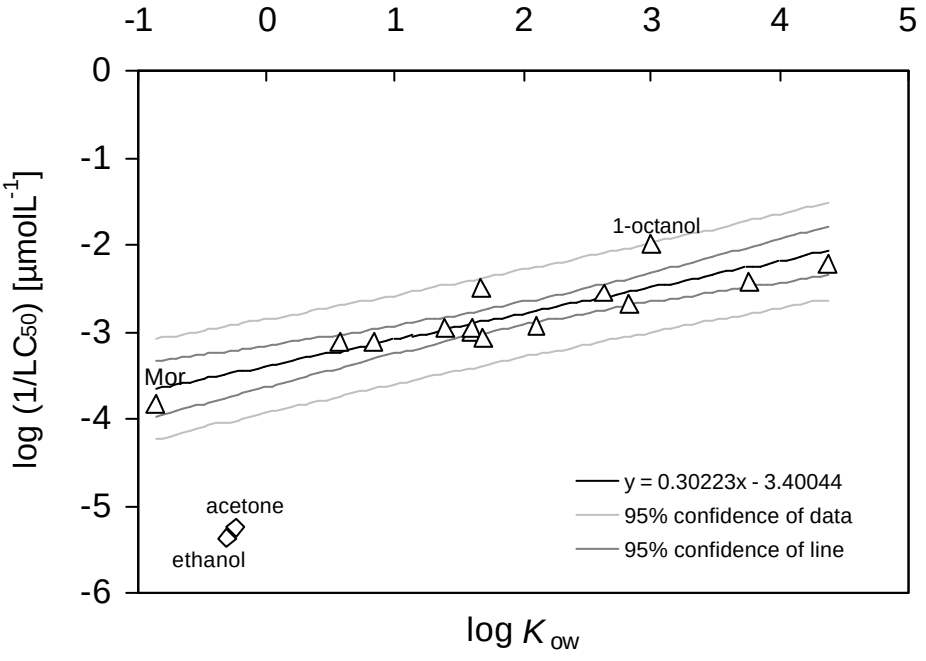


Figure 26: Relationship between toxicity and lipophilicity of non-polar narcotics found in the embryotest with zebrafish (Mor = morpholine).

The toxicity of seven anilines, six phenols and two benzoic acid compounds (Schulte, 1997), and the toxicity of *p*-tert-butylphenol (Maiwald, 1997) were tested with the embryotest. Anilines and phenols are also considered to be polar narcotics (Veith, and Broderius, 1987; Schultz *et al*., 1989 and 1991a; Nendza and Russom, 1991; Verhaar *et al.*, 1992). In the following the benzoic acid compounds will be included to the group of narcotic acting anilines and phenols due to their similarity.

For polar narcotics such as the primary amines tested within this study, the anilines, the phenols, and the benzoic acids the relationship between the toxicity and their lipophilicity should be studied. The regression yields:

$$log\ (1/LC50) = 0.578\ log\ K_{ow} - 3.467 \quad (n = 29;\ R^2 = 0.73;\ r = 0.85)$$

Freidig and Hermens (2000) mentioned that such compounds having a $T_e > 5$ are considered to be „reactive chemicals“ and such with a $T_e < 5$ are narcotics. For this dataset the excess toxicity as defined by Lipnick (1991) can be used to establish this criteria for effect-based separation. If ones compare the excess toxicity (T_e) the observed toxicity of five compounds is higher than expected (Table 18). Thus, 4-aminophenol was approximately 400 times, 2,4-dinitrophenol 106 times and hydrochinone 24 times more toxic then predicted. For the compounds 4-nitrophenol and 3,4-dichloroaniline the T_e was slightly greater than 5.

If these five outliers are omitted from regression analysis a significant relationship between toxicity and lipophilicity can be found (Figure 27):

$$log\ (1/LC50) = 0.544\ log\ K_{ow} - 3.467 \quad (R^2 = 0.82;\ r = 0.90;\ Q^2 = 0.77\ [LSO]$$

Table 18: Excess toxicity (T_e) for polar narcotics. Observed LC_{50} – values were obtained from several sources on the basis of experimental results using the embryotest with *Danio rerio*. Predicted LC_{50} – values were calculated using the model regression: $log\ (1/LC50) = 0.578\ log\ K_{ow} - 3.467$. T_e was calculated according to equation 8 (chapter 3.5.1). $T_e > 5$ are highlighted.

Substance	**LC_{50} (observed)**	**LC_{50} (predicted)**	**T_e**
Primary amines [a]			
n-Propylamine	1,339	1,547	1.2
Isopropylamine	2,531	2,074	0.8
n-Butylamine	491	806	1.6
sec-Butylamine	1,301	1,109	0.9
Isobutylamine	1,267	1,095	0.8
n-Pentylamine	354	403	1.1
Isopentylamine	678	555	0.8
Cyclohexylamine	639	403	0.6
n-Hexylamine	418	189	0.5
n-Heptylamine	247	96	0.4
n-Octylamine	197	62	0.3
n-Nonylamine	80	37	0.5
n-Decylamine	20	19	1.0
Anilines [b]			
3,4-Dichloroaniline	15	82	**5.4**
2,4-Dichloroaniline	123	72	0.6
4-Chloroaniline	194	257	1.3
3- Chloroaniline	138	240	1.7
2- Chloroaniline	208	234	1.1
2- Nitroaniline	177	250	1.4
Aniline	1,560	885	0.6
Phenols [c]			
Phenol	604	420	0.7
4-Nitrophenol	40	231	**5.8**
4-Chlorophenol	278	122	0.4
4-Aminophenol	6	2,779	**463.2**
2,4-Dinitrophenol	3	317	**105.8**
Hydroquinone	56	1,337	**23.9**
p-tert-Butylphenol [d]	11	36	3.3
Benzoic acids [c]			
Benzoic acid	232	243	1.0
4-Nitrobenzoic acid	154	237	1.5

[a] this study
[b,c] from Schulte (1997)
[d] from Maiwald (1997)

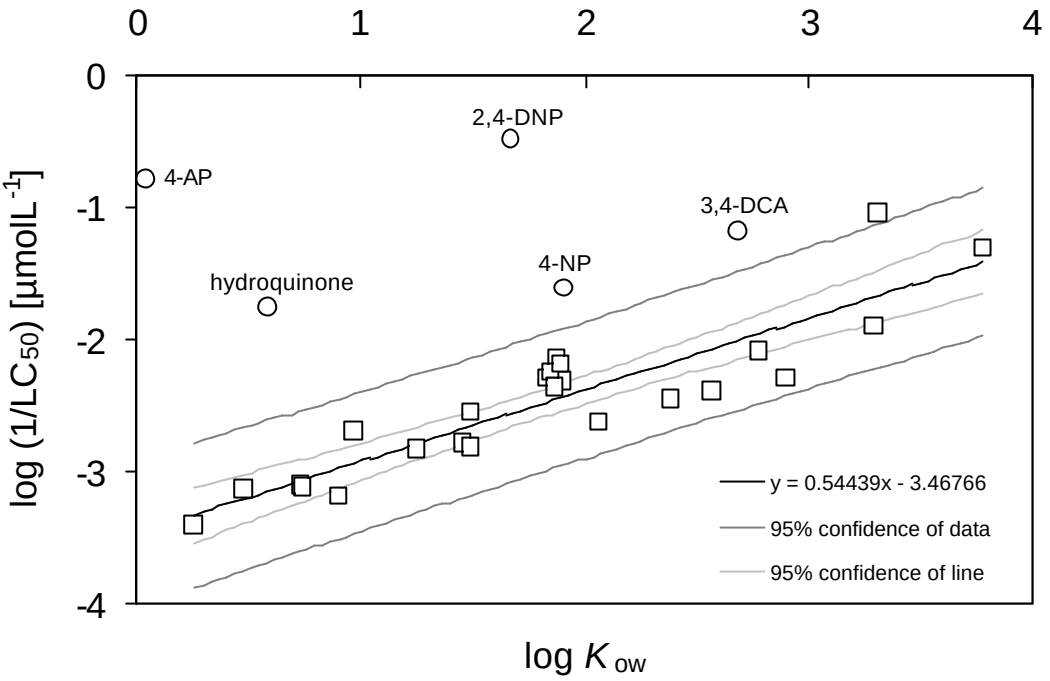


Figure 27: Relationship between toxicity (LC_{50}) and lipophilicity (log K_{ow}) of polar narcotics. Outliers were not included in regression. (4-AP = 4-aminophenol, 2,4-DNP = 2,4-dinitrophenol, 4-NP = 4-nitrophenol, 3,4-DCA = 3,4-dichloroaniline)

Schulte (1997) observed in the case of 2,4-DNP, 4-AP and hydroquinone that the period of gastrula was not finished within the embryonic development. Embryos exposed to 4-nitrophenol showed a hyperblastula which means that the stage of gastrula was not reached. These findings indicate a specific mode of action. Nendza (1998) mentioned that chemicals containing functional groups such as quinone, polynitroaromatic and imidazole are reactive chemicals. Some reactive compounds act as electrophiles and react with nucleophilic groups such $–NH_2$, OH or SH of physiological macromolecules such as proteins and DNA bases (Cronin and Dearden, 1995). Polynitroaromatics, such as 2,4-dinitrophenol, are known to act as uncouplers of oxidative phosphorylation (Nendza, 1998). Schüürmann and Segner (1994) suggest that the toxicity of phenols increases with increasing acidity of their phenolic group. The acidity for 2,4-DNP is with a pK_a of 4.1 higher than that of 4-nitrophenol with a pK_a of 7.21.

Finally, using the literature data (see Table 6) the toxicity of aliphatic amines on the embryos of zebrafish should be compared with data from other aquatic species. As aforementioned most data were available for the subclass of primary aliphatic amines. In Table 19 the regression models for the relationship between toxicity and lipophilicity as descriptor for *P. promelas*, *Daphnia magna*, and for the embryos of *Danio rerio* are compiled. For this compilation data for of least seven primary aliphatic amines in one species were required for statistical reasons. Further, data found for the ciliate *Tetrahymena pyriformis* were included. These data were taken from Schultz *et al.* (1991b) who evaluated the toxicity of several amines in the 48h static population growth impairment assay with *T. pyriformis*.

Table 19: Regression of toxicity (as log (1//LC_{50}) on lipophilicity (as log K_{ow}) for the subclass of primary amines on different testorganisms.

Testorganism	***n***	**Regression model**	**R^2**	**r**
Embryo of *Danio rerio* [a]	13	log (1/LC_{50}) = 0.467*log K_{ow} – 3.43	0.91	0.95
Pimephales promelas [b]	9	log (1/LC_{50}) = 0.898*log K_{ow} – 4.38	0.97	0.98
Daphnia magna [c]	9	log (1/EC_{50}) = 0.778*log K_{ow} – 3.67	0.96	0.98
Tetrahymena pyriformis [d]	9	log (1/IGC_{50}) = 0.819*log K_{ow} – 4.58	0.89	0.94

[a] data from this study
[b] data from Brooke *et al.* (1984), Geiger *et al.* (1988; 1990), Newsome *et al.* (1991), and Broderius *et al.* (1995),
[c] data from Calamari *et al.* (1980) and Pederson *et al.* (1998)
[d] data from Schultz *et al.* (1991b)

The regression models for *P. promelas* and *T. pyriformis* are very similar and Schultz *et al.* (1991b) mentioned that the IGC_{50} of the *Tetrahymena* system is a good predictor for the *Pimephales* system as found for a dataset of 23 aliphatic amines and aromatic amines. The toxicity of primary amines for the embryos of zebrafish and *Daphnia magna* are also similar, but the slope of the regression model for the *Daphnia* system is higher by a factor of 1.7. Further, the regression for the zebrafish embryo is not as steep compared to the three other regressions (Figure 28).

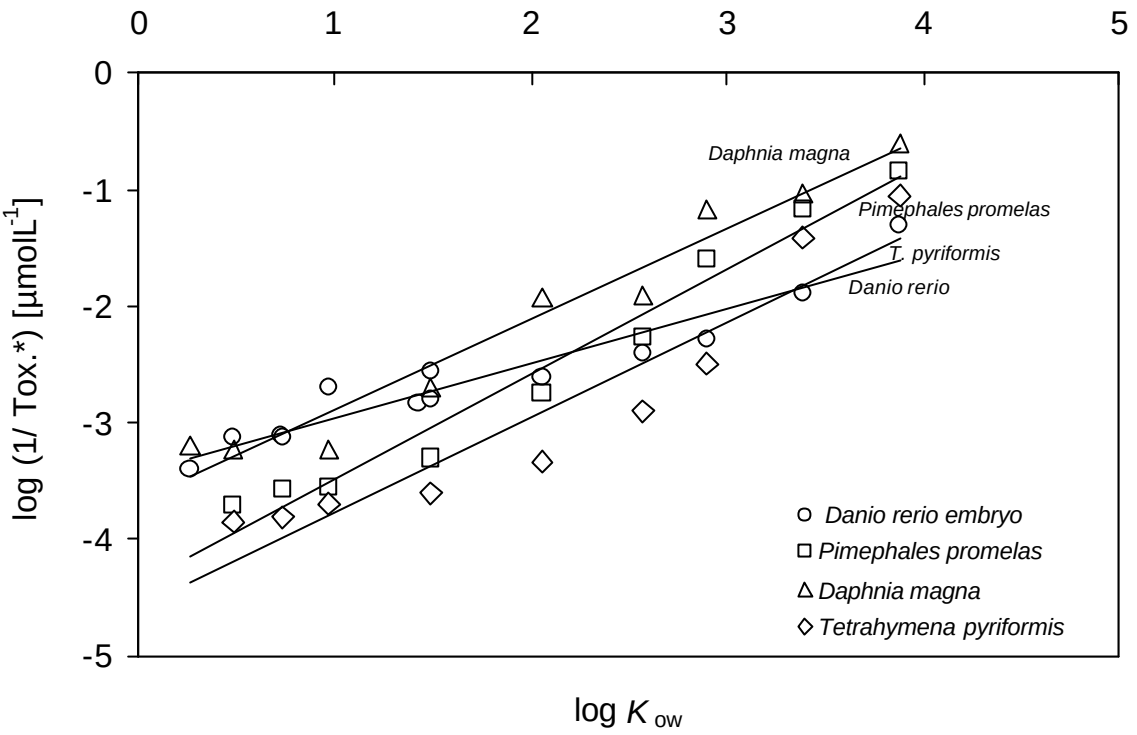


Figure 28: Comparison of the relationship between toxicity data and lipophilicity determined for primary aliphatic amines with different aquatic species. (Tox* corresponds to: LC_{50} for *P. promelas* and embryos of *Danio rerio*; EC_{50} for *Daphnia magna*, and IGC_{50} (median impairment growth concentration) for *T. pyriformis*)

Nevertheless, a correlation matrix showed that the toxicity of each system can be well described by one of the others (Table 20) if the common data for primary amines on the respective testorganism are used.

Table 20: Correlation matrix of toxicity (given as log ($1/LC_{50}$ or EC_{50} or IGC_{50}) of primary amines on *Danio rerio* (embryo), *Pimephales promelas*, *Daphnia magna* and *Tetrahymena pyriformis*.

	LC_{50} embryo	LC_{50} *Pimephales promelas*	EC_{50} *Daphnia magna*	IGC_{50} *Tetrahymena pyriformis*
LC_{50} embryo	1	0.92	0.91	0.95
LC_{50} *Pimephales promelas*	0.92	1	0.98	0.97
EC_{50} *Daphnia magna*	0.91	0.98	1	0.92
IGC_{50} *Tetrahymena pyriformis*	0.95	0.97	0.92	1

3.5.2 Structure-Dose Relationships

In the latter chapter the exposure of aliphatic amines via the waterphase with the resulting LC_{50} was discussed. But as a rule the dose which affects the biota is the better way to describe the toxicity of chemicals. Therefore, structure-dose relationships were performed. The lethal dose of aliphatic amines is given as the log ($1/LD_{50}^{*}$) which is the logarithm of the inverse of the 48 h 50% mortality dose (µmolkg^{-1}] for the embryos of zebrafish. The LD_{50}^{*} for each compound was calculated on the basis of the experimental BCF of ^{14}C-butylamine and the correlation between the lipophilicity and BCF´s including the data from Ensenbach (1987) and the experimentally derived LC_{50} - values (see chapter 3.4).

In Figure 29 the relationship between the lethal doses and the lipophilicity as descriptor of the 36 investigated aliphatic amines is shown. No significant correlation could be found, and the regression coefficient was only $R^2 = 0.35$ and the $Q^2 = 0.18$ using the „leave several out“ [LSO] method.

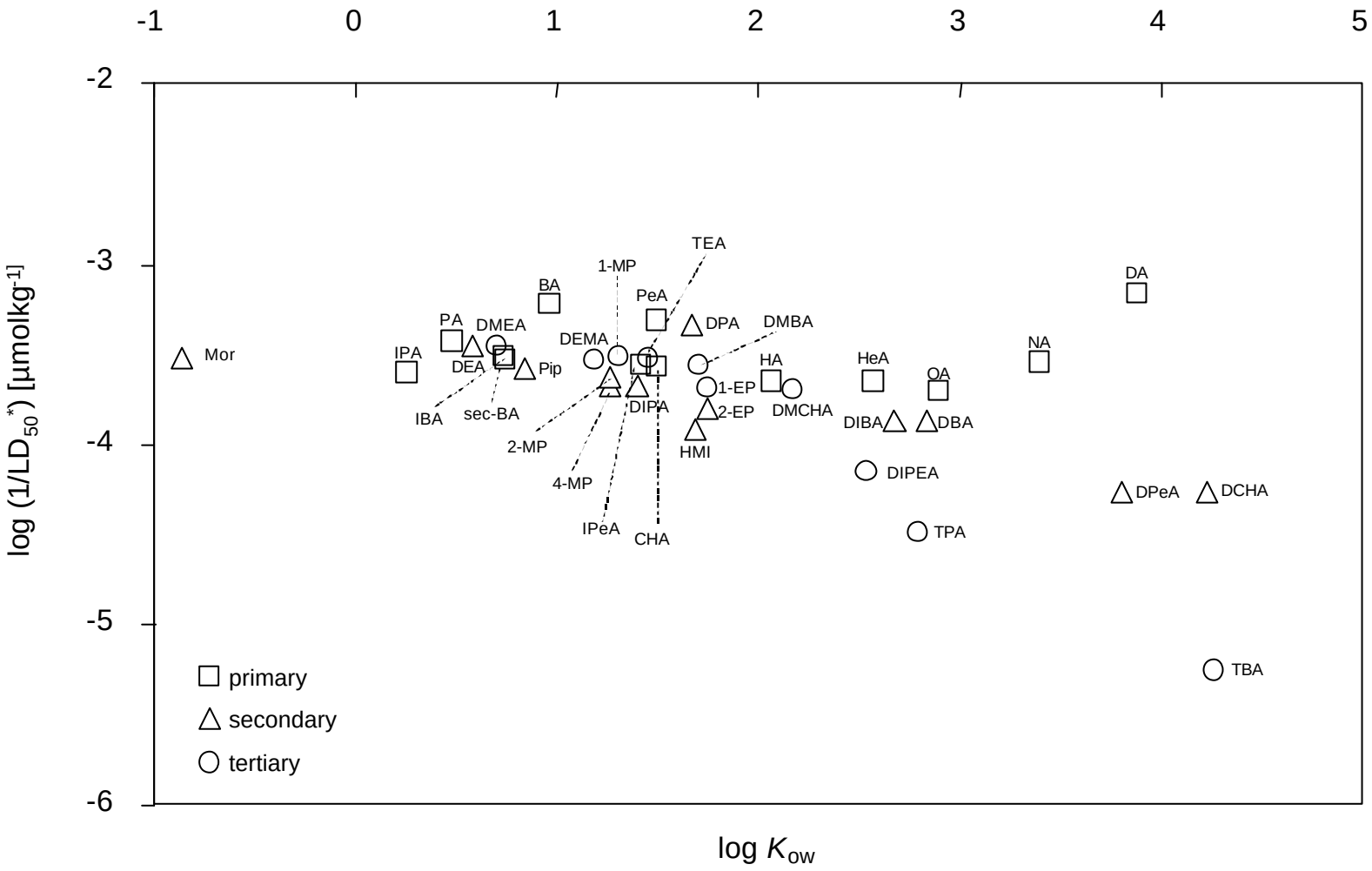


Figure 29: Relationship between the dose (log $1/LD_{50}^{*}$) and lipophilicity (log K_{ow}) for aliphatic amines in the embryo of *Danio rerio*.

(A = amine; PA propyl-; IPA isopropyl-, BA butyl-, IBA isobutyl-, sec-BA sec-butyl-, PeA isopentyl-, IpeA isopentyl-, CHA cyclohexyl-, HA hexyl-, HeA heptyl-, NA nonyl-, DA decyl-; DEA ditehyl-, DPA dipropyl-, DIPA diisopropyl-, DBA dibutyl-, DIBA diisobutyl-,DPeA dipentyl-, Pip piperidine, Mor morpholine, 2-MP 2-methylpiperidine, 4-MP 4-methylpiperidine, 2-EP 2-ethylpiperidine, HMI hexamethyleneimine, DCHA dicyclohexylamine; TEA triethyl-, TPA tripropyl-, TBA tributyl-, DMCHA dimethylcyclohexyl-, DMEA dimethylethyl-, DEMA diethylmethyl-, DMBA dimethylbutyl-, DIPEA diisopropylethyl-, 1-MP 1-methylpiperidine, 1-EP 1-ethylpiperidine)

Viewed separately, for the subclass of primary aliphatic amines also no significant relationship of the dose and lipophilicity could be found (R^2 = 0.0018; F = 0.02; P = 0.889) as to be seen in Figure 30, which means that the dose affecting lethality in the embryos is comparable for all compounds of this subclass.

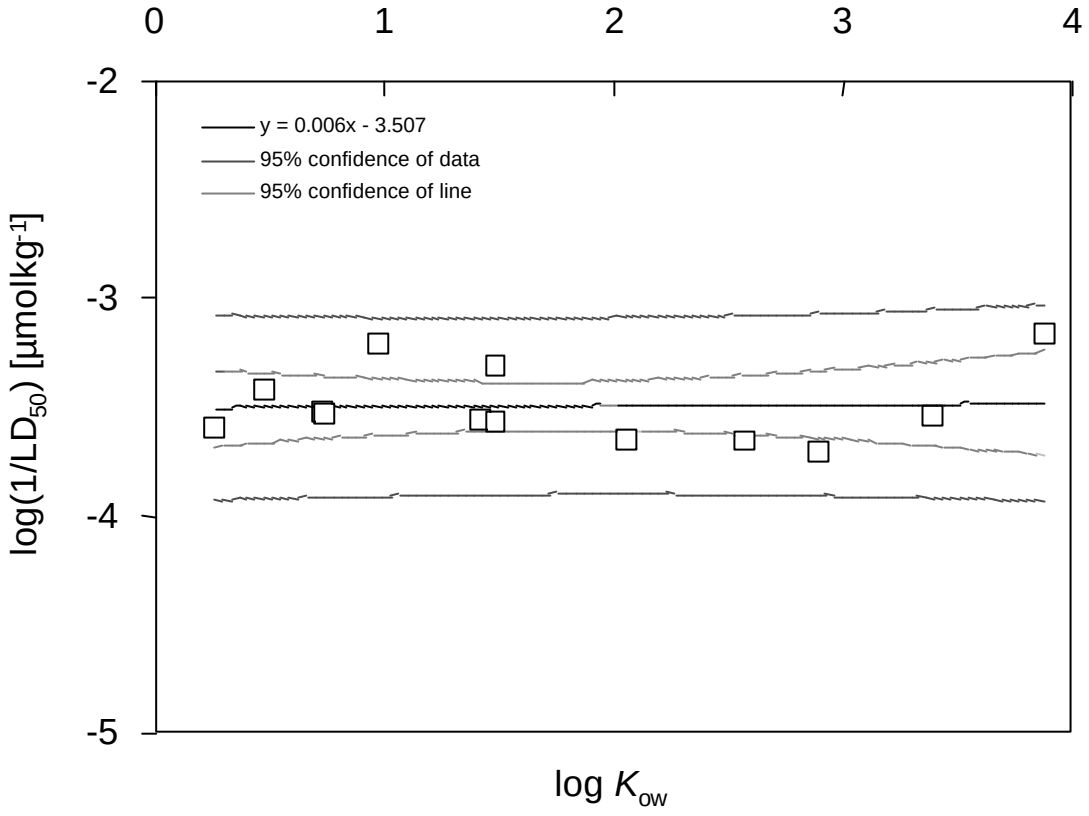


Figure 30: Relationship between the dose (log $1/LD_{50}^{*}$) and lipophilicity (log K_{ow}) for primary aliphatic amines (n = 13) in the embryo of *Danio rerio*.

At first glance for the subclass of secondary aliphatic amines the lethal doses can be described significantly better by their lipophilicity (Figure 31) with a regression coeffiecient of R^2 = 0.69 (F = 25.13; P = 0.00039). However, under the assumption that the toxicity does not depend on the lipophilicity dipentylamine and dicyclohexylamine were omitted from the data set. Thus, the recalculated regression with R^2 = 0.30 (F = 5.39; P = 0.046) showed that the lethal dose is comparable for the remaining compounds of this subclass.

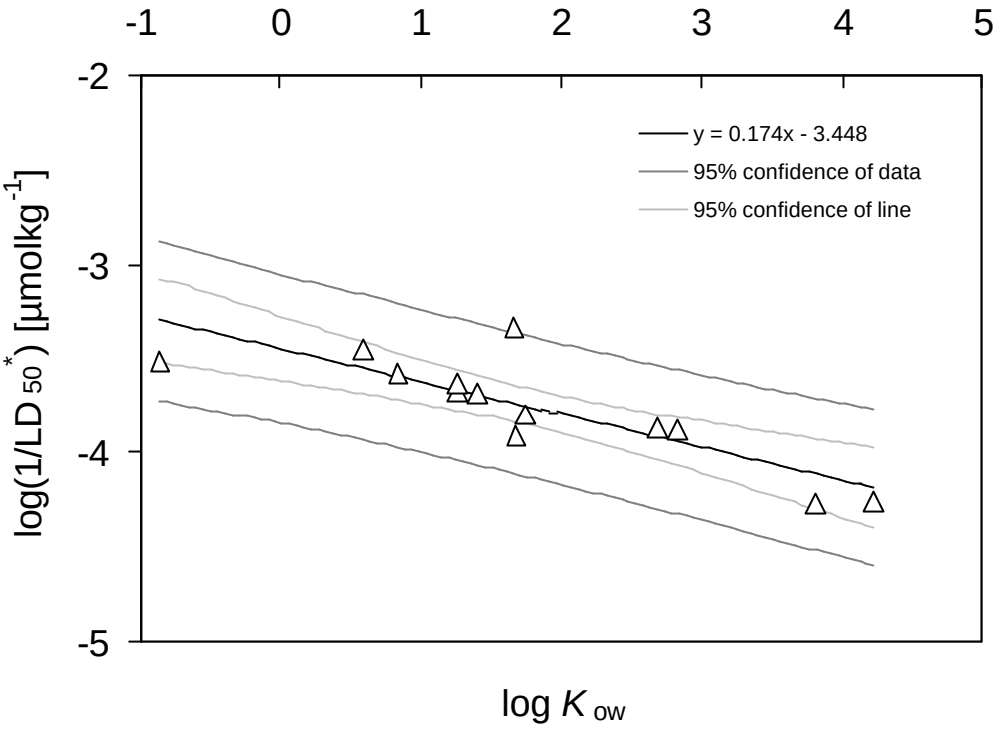


Figure 31: Relationship between the dose (log $1/LD_{50}^{*}$) and lipophilicity (log K_{ow}) for secondary aliphatic amines (n = 13) in the embryo of *Danio rerio*.

A significant regression with $R^2 = 0.87$ ($F = 92.96$; $P < 0.00001$) between the lethal doses and the lipophilicity of test compounds was found for the subclass of the tertiary aliphatic amines (Figure 32), whereas the relationship between lipophilicity and LC_{50} could only be described by a bilinear regression model. But the linearity seems to depend on three compounds: diisopropylethylamine, tripropylamine and tributylamine. Therefore, this three amines were omitted from the data set. The recalculated regression is even significant with $R^2 = 0.78$ ($F = 21.43$; $P = 0.006$), but as can be seen in tendency the lethal dose is comparable for the remaining compounds of this subclass.

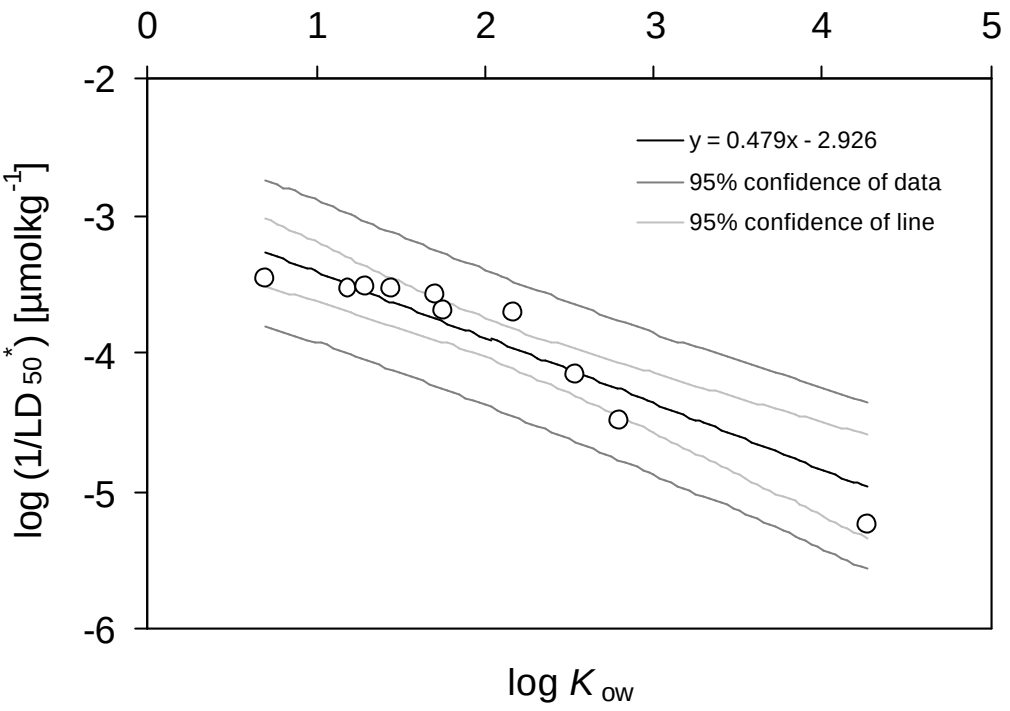


Figure 32: Relationship between the dose (log $1/LD_{50}^{*}$) and lipophilicity (log K_{ow}) for tertiary aliphatic amines (n = 10) in the embryo of *Danio rerio*.

Of course, the relationships between lethal doses and lipophilicity for aliphatic amines discussed here are based on calculated LD_{50} values but nevertheless, it can give an impression about the acting dose of the corresponding amine in the embryos of zebrafish.

A comparison of the LD_{50}^{*}´s calculated for the embryos of zebrafish with data derived from the rat oral toxicity assay was performed. The data for rat oral toxicity were taken from Greim *et al*. (1998), Jäckel and Klein (1991) and other sources (see also Table 6). The differences of the toxicity between embryos of zebrafish and the rat can be calculated using a so-called „toxic ratio“ (T_{ratio}) (Table 21).

Table 21: Compilation of toxicity data for LD_{50}^{*} [*Danio rerio* embryo] and LD_{50} [rat oral], their "toxic ratio" [T_{ratio}] for several aliphatic amines. A T_{ratio} greater than 3 and smaller than 0.3 is highlighted.

Substance	**LD_{50} [rat oral]**	**LD_{50}^{*} [*D. rerio* embryo]**	**T_{ratio}**
Propylamine	7,952	2,664	2.9
Isopropylamine	7,520	3,973	1.9
Butylamine	5,004	1,640	3.0
Isobutylamine	3,063	3,282	0.9
sec-Butylamine	7,451	3,409	2.2
Cyclohexylamine	3,882	3,706	1.0
Hexylamine	6,621	4,443	1.5
Decylamine	1,780	1,468	1.2
Diethylamine	7,383	2,818	2.6
Dipropylamine	6,869	2,165	**3.2**
Diisopropylamine	4,941	4,773	1.0
Dibutylamine	2,859	7,534	0.4
Diisobutylamine	1,996	7,493	**0.3**
Dipentylamine	1,716	18,531	**0.1**
Piperidine	5,285	3,774	1.4
Morpholine	12,052	3,312	**3.6**
Hexamethyleneimine	4,134	8,257	0.5
Dicyclohexylamine	1,580	18,301	**0.09**
Triethylamine	4,546	3,325	1.4
Tripropylamine	503	30,406	**0.02**
Tributylamine	2,913	178,425	**0.02**
N,N-Dimethylcyclohexylamine	3,922	4,983	0.8
Dimethylethylamine	8,287	2,844	2.9
1-Ethylpiperidine	3,212	4,820	0.7

A comparison showed that within the primary aliphatic amines the toxicity is similar or differs by a factor of three only. Within the subclasses of secondary and tertiary amines the toxicity differs greatly. Within the subclass of secondary aliphatic amines the toxicity of dipropylamine and morpholine is by a factor of 3.2 and 3.6 greater, whereas diisobutylamine was three times less toxic on the rat than on the embryos of zebrafish. Dipentylamine and dicyclohexylamine were by a factor of approximately 11 even more toxic to the rat. Within

the tertiary amines the toxicity of tripropylamine and tributylamine on the embryo of zebrafish is by a factor of 60 lower than that on the rat. These 7 compounds were omitted from the data set, and the remaining were used to compare the rat toxicity data with the *D. rerio* toxicity data (Figure 33).

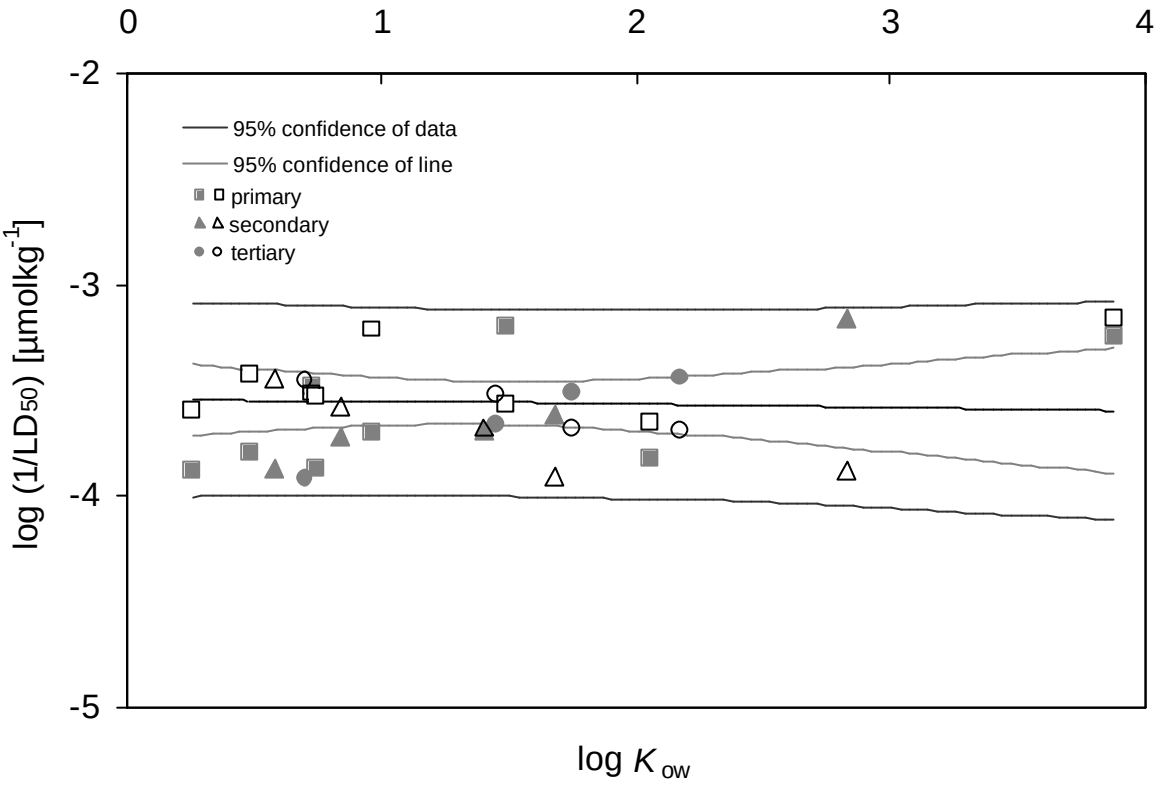


Figure 33: Relationship between the dose (log $1/LD_{50}$) and lipophilicity (log K_{ow}) using rat oral toxicity (filled symbols) and *D. rerio* embryo toxicity data (empty symbols) for aliphatic amines (omitted are: dipropylamine, diisobutylamine, dipentylamine, dicyclohexylamine, morpholine, tripropylamine, and tributylamine).

As can be seen in Figure 33 no relationship between toxicity of 17 aliphatic amines on the embryos of zebrafish and the lipophilicity could be found (R^2 = 0.0053; F = 0.08; P = 0.78). The rat oral toxicity data lay within the 95 % confidence band of the toxicity data of the *D. rerio* embryos which means that the toxicity of the rat is comparable to that of the embryos of zebrafish.

4 SUMMARY

In this study the toxicity of 13 primary, 13 secondary and 10 tertiary aliphatic amines on the embryos of zebrafish using the *Dar*T (*Danio rerio* toxicity assay) was investigated.

Defined lethal and sublethal effects were recorded within 48h of embryonic development. Coagulated embryos and such without a heartbeat were determined as lethal effects and resulting LC_{50}-values were calculated. The observed sublethal effects such as hypopigmentation, yolk sack and pericard oedema, and effects on the sacculus with the otoliths were covered by the lethal effects, and therefore valid EC_{50}-values for sublethal effects could not be calculated.

QSARs calculated for predicting the toxicity showed that the whole group of aliphatic amines can not be described by the lipophilicity (log K_{ow}) alone. For this dataset a satisfactory multiple regression model using the lipophilicity, the effective diameter (D_{eff}) and the maximum bond on hydrogen atom (H^{+}_{max}) could be found.

For the subclass of the primary and secondary aliphatic amines a good relationship between the toxicity and the lipophilicity could be found. The toxicity of the compounds increased with increasing lipophilicity. Including other descriptors in multiple regression analysis did not improve statistics.

The toxicity of the subclass of tertiary aliphatic amines could be described best by a bilinear regression model including the lipophilicity. Tributylamine was omitted from this analysis because of unreliable experimental results. The toxicity increased up to a log K_{ow} of approximately 2 and then decreased with increasing lipophilicity. No multiple regressions with a $R^2 > 0.5$ could be found. To accept or to reject the bilinear relationship three to four further tertiary amines with an log K_{ow} between 3 and 4 should be tested within an embryotest.

The multiple regression model gained for all aliphatic amines was used to determine the predicted toxicity. The so-called excess toxicity (T_e) was calculated which is the quotient of the predicted and the observed toxicity. The T_e-values showed that the observed toxicity of the primary decylamine was three times lower then expected. For the secondary dibutylamine and triethylamine the predicted toxicity was three times lower and two times higher, respectively.

QSARs for modelling the fish toxicity were performed and found that the primary aliphatic amines as well as anilines and phenols act as polar narcotics. The secondary and tertiary aliphatic amines are defined as acting by non-polar narcosis.

Including toxicity data of the subclass of primary amines and of anilines and phenols a satisfactory relationship between toxicity and lipophilicity with $R^2 = 0.82$ could be found. Within this context five compounds having a $T_e > 5$ were identified and excluded from the regression. These compounds act by a specific mode of action.

The addition of toxicity data of three non-polar acting alcohols to the dataset of the secondary amines did not improve the relationship between toxicity and lipophilicity, especially acetone and ethanole must be omitted from regression.

Regression models found for the toxicity of primary aliphatic amines in different aquatic biota, such as the fathead minnow *Pimephales promelas*, *Daphnia magna* and *Tetrahymena pyriformis* were compared with those found for the embryos of zebrafish. In general, the toxicity of each species was found to be a good predictor for each other. However, the slope of the regression model for the toxicity on the embryos of zebrafish was less steep then those for the other three species.

Further, the bioconcentration of ^{14}C radiolabeled butylamine in the eggs of zebrafish was studied until the steady state of 24 h. Lethal doses (LD_{50}^*) could be calculated by using BCFs determined in the eggs for other compounds.

For the primary aliphatic amines no relationship between LD_{50}^* and lipophilicity was found. The same trend was found for the secondary and the tertiary aliphatic amines if five secondary and two tertiary amines were omitted from the data set which means that the lethal doses of the remaining amines are comparable.

These data were compared with available rat oral toxicity data. The comparison showed that within the primary amines the toxicity is similar or differs by a factor of three only. Within the subclasses of secondary and tertiary amines the toxicity differs greatly. For five secondary and two tertiary differences by a factor of three to 60 could be found. Nevertheless, if these data are omitted the rat oral toxicity lays within the same range of that of the toxicity for the embryos of *Danio rerio* for the remaining amines.

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6 APPENDIX

Table A 1: CAS-No., simply molecular input line entry system-code (SMILES), toxicity and molecular descriptors of aliphatic amines

Substance	**CAS-No.**	**SMILES**	**log K_{ow} (est.)**	**log K_{ow} (exp.)**	**log K_{ow} (adj.)**	**LC_{50} [µmolL^{-1}]**	**log (1/LC_{50})**
Propylamine	107-10-8	NCCC	0.34	0.48	0.48	1,339	-3.13
Isopropylamine	75-31-0	NC(C)C	0.27	0.26	0.26	2,531	-3.40
Butylamine	109-73-9	NCCCC	0.83	0.97	0.97	491	-2.69
Isobutylamine	78-81-9	NCC(C)C	0.76	0.73	0.73	1,267	-3.10
sec-Butylamine	13952-84-6	NC(CC)C	0.76	0.74	0.74	1,301	-3.11
Pentylamine	110-58-7	NCCCCC	1.33	1.49	1.49	354	-2.55
Isopentylamine	107-85-7	NCCC(C)C	1.25	1.25	1.41	678	-2.83
Cyclohexylamine	108-91-8	NC(CCCC1)C1	1.63	1.49	1.49	639	-2.81
Hexylamine	111-26-2	NCCCCCC	1.82	2.06	2.06	418	-2.62
Heptylamine	111-68-2	NCCCCCCC	2.31	2.57	2.57	247	-2.39
Octylamine	111-86-4	NCCCCCCCC	2.8	2.9	2.9	197	-2.29
Nonylamine	112-20-9	NCCCCCCCCC	3.29	3.29	3.39	80	-1.90
Decylamine	2016-57-1	NCCCCCCCCCC	3.78	3.78	3.88	20	-1.30
Diethylamine	109-89-7	N(CC)CC	0.81	0.58	0.58	1,275	-3.11
Dipropylamine	142-84-7	N(CCC)CCC	1.79	1.67	1.67	308	-2.49
Diisopropylamine	108-18-9	N(C(C)C)C(C)C	1.64	1.4	1.4	904	-2.96
Dibutylamine	111-92-2	N(CCCC)CCCC	2.77	2.83	2.83	473	-2.67
Diisobutylamine	110-96-3	N(CC(C)C)CC(C)C	2.63	2.63	2.69	365	-2.56
Dipentylamine	2050-92-2	N(CCCCC)CCCCC	3.76	3.76	3.82	272	-2.43
Piperidine	110-89-4	N(CCCC1)C1	1.19	0.84	0.84	1,297	-3.11
Morpholine	110-91-8	O(CCNC1)C1	-0.56	-0.86	-0.86	6,901	-3.84
2-Methylpiperidine	109-05-7	N(C(CCC1)C)C1	1.61	1.61	1.26	1,032	-3.01
4-Methylpiperidine	626-58-4	N(CCC(C1)C)C1	1.61	1.61	1.26	937	-2.97
2-Ethylpiperidine	1484-80-6	N(C(CCC1)CC)C1	2.1	2.1	1.75	830	-2.92
Hexamethyleneimine	111-49-9	N(CCCCC1)C1	1.68	1.68	1.68	1,163	-3.07
Dicyclohexylamine	101-83-7	N(C(CCCC1)C1)C(CCCC2)C2	4.37	4.37	4.23	172	-2.24
Triethylamine	121-44-8	N(CC)(CC)CC	1.51	1.45	1.45	598	-2.78
Tripropylamine	102-69-2	N(CCC)(CCC)CCC	2.99	2.79	2.79	1,318	-3.12
Tributylamine	102-82-9	N(CCCC)(CCCC)CCCC	4.46	4.46	4.26	1,625	-3.21
Dimethylcyclohexylamine	98-94-2	N(C(CCCC1)C1)(C)C	2.31	2.31	2.17	417	-2.62
Dimethylethylamine	598-56-1	N(CC)(C)C	0.53	0.7	0.7	1,133	-3.05
Diethylmethylamine	616-39-7	N(CC)(CC)C	1.02	1.02	1.19	803	-2.90
Dimethylbutylamine	927-62-8	N(CCCC)(C)C	1.51	1.7	1.7	504	-2.70
Diisopropylethylamine	7087-68-5	N(C(C)C)(C(C)C)CC	2.35	2.35	2.54	809	-2.91
1-Methylpiperidine	626-67-5	N(CCCC1)(C1)C	1.4	1.3	1.3	689	-2.84
1-Ethylpiperidine	766-09-6	N(CCCC1)(C1)CC	1.89	1.75	1.75	630	-2.80

Table A 1: (Continued)

Substance	**pK_a**	**N- Gasteiger**	**e_{HOMO}**	**e_{LUMO}**	**DIFF**	**Hardness**	**EN**	**HOF**	**Dipol**	**D_{max}**
Propylamine	10.7	-0.33141	-9.47	3.15	12.62	6.31	3.16	-17.67	1.45	6.87
Isopropylamine	10.6	-0.3292	-9.38	2.98	12.36	6.18	3.2	-17.33	1.32	6.74
Butylamine	10.8	-0.3314	-9.38	2.96	12.34	6.17	3.21	-21.90	1.34	9.09
Isobutylamine	10.7	-0.33115	-9.46	3.11	12.56	6.28	3.17	-23.33	1.45	7.39
sec-Butylamine	10.6	-0.32895	-9.48	3.02	12.49	6.25	3.23	-23.40	1.37	7.72
Pentylamine	10.6	-0.3314	-9.39	2.94	12.33	6.16	3.22	-27.32	1.33	10.3
Isopentylamine	10.6	-0.33139	-9.47	3.05	12.52	6.26	3.21	-28.78	1.41	8.65
Cyclohexylamine	10.6	-0.32868	-9.38	2.91	12.29	6.15	3.24	-19.90	1.3	8.09
Hexylamine	10.6	-0.3314	-9.5	3	12.50	6.25	3.25	-34.13	1.45	11.1
Heptylamine	10.7	-0.3314	-9.39	2.94	12.33	6.16	3.22	-37.98	1.32	11.7
Octylamine	10.7	-0.3314	-9.39	2.91	12.31	6.15	3.24	-43.58	1.33	14.1
Nonylamine	10.6	-0.3314	-9.5	2.97	12.47	6.24	3.26	-50.39	1.46	14.9
Decylamine	10.6	-0.3314	-9.39	2.91	12.30	6.15	3.24	-54.43	1.33	16.6
Diethylamine	11.1	-0.318	-9.14	2.76	11.90	5.95	3.19	-19.58	1.19	9.16
Dipropylamine	11	-0.3175	-9.17	2.76	11.93	5.97	3.2	-31.01	1.25	10.9
Diisopropylamine	11.1	-0.31311	-9.22	2.68	11.90	5.95	3.27	-33.11	1.23	9.03
Dibutylamine	11.4	-0.31749	-9.17	2.65	11.82	5.91	3.26	-41.05	1.15	14.2
Diisobutylamine	10.9	-0.317	-9.13	2.84	11.97	5.98	3.15	-42.21	1.35	10.6
Dipentylamine	11.2	-0.31749	-9.18	2.69	11.87	5.93	3.24	-51.38	1.18	14.5
Piperidine	11.3	-0.31749	-9.16	2.86	12.01	6.01	3.15	-16.52	1.18	7.15
Morpholine	8.49	-0.37785	-9.38	2.59	11.96	5.98	3.39	-43.07	1.23	6.77
2-Methylpiperidine	11.1	-0.31504	-9.26	2.89	12.15	6.08	3.19	-20.92	1.34	8.17
4-Methylpiperidine	11.1	-0.31747	-9.24	2.94	12.19	6.09	3.15	-20.78	1.44	7.42
2-Ethylpiperidine	11.1	-0.31479	-9.13	2.81	11.94	5.97	3.16	-27.44	1.12	9.64
Hexamethyleneimine	11.1	-0.31749	-9.14	2.96	12.10	6.05	3.09	-19.59	1.39	7.63
Dicyclohexylamine	10.4	-0.31207	-9.18	2.62	11.81	5.9	3.28	-47.89	1.17	12.4
Triethylamine	10.8	-0.30468	-9.01	2.56	11.57	5.78	3.23	-26.57	1.01	8.28
Tripropylamine	10.7	-0.30393	-9.04	2.5	11.53	5.77	3.27	-43.15	0.97	10.7
Tributylamine	10.9	-0.3039	-9.04	2.47	11.51	5.76	3.28	-58.87	0.95	12.6
Dimethylcyclohexylamine	10.6	-0.30699	-8.97	2.59	11.56	5.78	3.19	-29.04	1.07	9.7
Dimethylethylamine	10.2	-0.31015	-9.04	2.66	11.71	5.85	3.19	-15.87	1.11	7.9
Diethylmethylamine	10.2	-0.30742	-9.03	2.55	11.58	5.79	3.24	-20.52	1.09	7.53
Dimethylbutylamine	10.2	-0.3099	-9.06	2.62	11.68	5.84	3.22	-26.78	1.09	10.4
Diisopropylethylamine	10.2	-0.29939	-8.85	2.35	11.19	5.6	3.25	-32.39	1.05	8.26
1-Methylpiperidine	10.1	-0.3069	-8.89	2.71	11.60	5.8	3.09	-16.68	1.21	8.1
1-Ethylpiperidine	10.1	-0.30416	-8.99	2.59	11.59	5.79	3.2	-24.30	1	9.48

Table A 1: (Continued)

Substance	**D_{eff}**	**D_{min}**	**SASA**	**SAVOL**	**V^+**	**V^-**	**V^{tot}**	**Q^+_{max}**
Propylamine	5.48	5.21	241.7	328.79	5.122	-4.796	9.918	0.2063
Isopropylamine	6.03	5.12	244.2	332.39	8.45	-7.302	15.752	0.2851
Butylamine	5.23	5.16	281.1	393.17	5.943	-5.176	11.119	0.2227
Isobutylamine	6.24	5.18	269.2	382.3	7.256	-7.018	14.274	0.2058
sec-Butylamine	5.96	5.36	271.1	383.46	6.607	-6.025	12.633	0.2248
Pentylamine	5.29	5.18	312.5	451.61	6.67	-5.968	12.638	0.2558
Isopentylamine	6.18	5.36	299.3	435.96	7.218	-6.914	14.131	0.2147
Cyclohexylamine	6.72	5.39	299.8	444.58	7.78	-6.88	14.659	0.2621
Hexylamine	5.35	5.23	343.3	507.7	6.043	-5.427	11.471	0.2086
Heptylamine	6.34	5.23	370.7	558.49	6.746	-6.329	13.075	0.2481
Octylamine	5.23	5.17	407.7	620.52	6.107	-5.721	11.828	0.2221
Nonylamine	5.24	5.17	439.2	678.89	5.613	-5.369	10.983	0.2042
Decylamine	5.23	5.17	471.1	735.44	5.814	-5.468	11.282	0.232
Diethylamine	5.24	5.15	283.9	396.03	5.08	-3.301	8.381	0.2241
Dipropylamine	5.59	5.38	345.1	508.38	5.131	-4.362	9.493	0.2176
Diisopropylamine	6.55	5.13	328	490.75	7.494	-6.003	13.496	0.2246
Dibutylamine	5.28	5.18	411	626.25	3.088	-2.32	5.408	0.2239
Diisobutylamine	6.54	6.34	375.3	588.17	6.407	-5.636	12.042	0.222
Dipentylamine	6.96	5.81	465.2	730.17	4.889	-4.579	9.467	0.2156
Piperidine	6.71	5.27	276.8	397.9	7.427	-5.669	13.096	0.2111
Morpholine	6.32	5.13	263.9	370.78	8.139	-5.814	13.954	0.2076
2-Methylpiperidine	6.64	5.7	301.8	448.28	6.535	-5.326	11.862	0.2709
4-Methylpiperidine	6.69	5.57	300.8	447.19	6.647	-5.91	12.557	0.2213
2-Ethylpiperidine	6.71	5.51	332.3	503.62	6.248	-4.933	11.181	0.2631
Hexamethyleneimine	6.65	5.39	296.5	440.47	5.884	-4.426	10.31	0.2189
Dicyclohexylamine	7.25	5.44	439.5	716.85	5.909	-5.06	10.969	0.2298
Triethylamine	7.93	5.42	328.7	488.49	6.596	-4.51	11.106	0.0911
Tripropylamine	9.86	5.48	425.9	665.58	5.849	-4.394	10.243	0.0823
Tributylamine	10.79	5.58	514.8	828.26	5.122	-4.059	9.181	0.0981
Dimethylcyclohexylamine	6.74	6.12	349.2	543.72	6.544	-5.231	11.775	0.1291
Dimethylethylamine	6.09	5.4	272.6	384.15	6.159	-3.913	10.072	0.1115
Diethylmethylamine	7.06	5.17	299.6	436.92	6.314	-4.424	10.738	0.0978
Dimethylbutylamine	6.11	5.46	336.7	499.61	4.746	-3.667	8.413	0.1297
Diisopropylethylamine	7.93	6.98	358	566.9	10.413	-8.631	19.043	0.4436
1-Methylpiperidine	6.72	5.57	302.3	449.66	6.013	-4.222	10.234	0.1194
1-Ethylpiperidine	6.71	5.5	332.6	502.85	5.688	-3.669	9.357	0.0891

Table A 1: (Continued)

Substance	Q^-_{max}	Q_{tot}	Q_{av}	H^+_{max}	**MW**	^0C	^1C	^2C
Propylamine	-0.5773	1.3377	0.1029	0.2063	59.11	3.414	1.914	1
Isopropylamine	-0.6188	2.3885	0.1837	0.2215	59.11	3.577	1.732	1.732
Butylamine	-0.6269	1.8309	0.1144	0.2227	73.13	4.121	2.414	1.354
Isobutylamine	-0.583	2.0343	0.1271	0.2058	73.13	4.284	2.27	1.802
sec-Butylamine	-0.6319	1.909	0.1193	0.2248	73.13	4.284	2.27	1.802
Pentylamine	-0.6273	2.2842	0.1202	0.2199	87.16	4.828	2.914	1.707
Isopentylamine	-0.5875	2.0872	0.1099	0.2147	87.16	4.992	2.77	2.183
Cyclohexylamine	-0.6297	2.3725	0.1186	0.2174	99.17	5.113	3.394	2.743
Hexylamine	-0.5743	2.356	0.1071	0.2086	101.19	5.536	3.414	2.061
Heptylamine	-0.6148	2.6118	0.1045	0.2159	115.22	6.243	3.914	2.414
Octylamine	-0.6281	2.8848	0.103	0.2216	129.24	6.95	4.414	2.768
Nonylamine	-0.5794	3.0344	0.0979	0.2042	143.27	7.657	4.914	3.121
Decylamine	-0.6281	3.3386	0.0982	0.2219	157.3	8.364	5.414	3.475
Diethylamine	-0.5497	1.9738	0.1234	0.2241	73.13	4.121	2.414	1.354
Dipropylamine	-0.582	2.3701	0.1077	0.2176	101.19	5.536	3.414	2.061
Diisopropylamine	-0.561	3.0199	0.1373	0.2246	101.19	5.862	3.126	3.023
Dibutylamine	-0.5982	2.3366	0.0834	0.2239	129.24	6.95	4.414	2.768
Diisobutylamine	-0.5465	2.7353	0.0977	0.222	129.24	7.276	4.126	3.719
Dipentylamine	-0.5647	3.3338	0.0981	0.2027	157.3	8.364	5.414	3.475
Piperidine	-0.5557	2.2394	0.1317	0.2111	85.15	4.243	3	2.121
Morpholine	-0.5275	2.1882	0.1459	0.2076	87.12	4.243	3	2.121
2-Methylpiperidine	-0.5556	2.2166	0.1108	0.2197	99.17	5.113	3.394	2.743
4-Methylpiperidine	-0.5367	2.1302	0.1065	0.2213	99.17	5.113	3.394	2.743
2-Ethylpiperidine	-0.5607	2.7561	0.1198	0.236	113.2	5.82	3.932	2.912
Hexamethyleneimine	-0.5213	1.7632	0.0882	0.2189	99.17	4.95	3.5	2.475
Dicyclohexylamine	-0.6372	3.628	0.1008	0.2298	181.32	8.933	6.449	5.244
Triethylamine	-0.3412	2.1967	0.0999	0.0911	101.19	5.699	3.346	2.091
Tripropylamine	-0.4083	2.6758	0.0863	0.0823	143.27	7.82	4.846	3.232
Tributylamine	-0.3655	3.0335	0.0758	0.0981	185.35	9.941	6.346	4.293
Dimethylcyclohexylamine	-0.3501	2.5007	0.0962	0.1291	127.23	6.69	4.305	3.642
Dimethylethylamine	-0.3177	1.7095	0.1068	0.1115	73.13	4.284	2.27	1.802
Diethylmethylamine	-0.3374	1.9835	0.1044	0.0978	87.16	4.992	2.808	1.922
Dimethylbutylamine	-0.3157	1.9002	0.0864	0.1297	101.19	5.699	3.27	2.536
Diisopropylethylamine	-0.462	4.1799	0.1493	0.1395	129.24	7.439	4.091	3.56
1-Methylpiperidine	-0.382	1.8545	0.0927	0.0868	99.17	5.113	3.394	2.743
1-Ethylpiperidine	-0.2921	1.9353	0.0841	0.0891	113.2	5.82	3.932	2.912

Table A 1: (Continued)

Substance	$^{3}C^{p}$	$^{3}C^{c}$	$^{0}C^{v}$	$^{1}C^{v}$	$^{2}C^{v}$	$^{3}C^{v,p}$	**MOLVOL**	**N - ESP**
Propylamine	0.5	0	2.992	1.615	0.789	0.289	21.491	-0.577
Isopropylamine	0	0.577	3.155	1.488	1.244	0	21.489	-0.619
Butylamine	0.707	0	3.699	2.115	1.142	0.558	26.691	-0.627
Isobutylamine	0.816	0.408	3.862	1.971	1.63	0.471	26.689	-0.583
sec-Butylamine	0.816	0.408	3.862	2.026	1.386	0.644	26.689	-0.632
Pentylamine	0.957	0	4.406	2.615	1.496	0.808	31.891	-0.627
Isopentylamine	0.866	0.408	4.569	2.471	1.971	0.744	31.889	-0.588
Cyclohexylamine	1.894	0.289	4.69	3.15	2.398	1.65	34.743	-0.63
Hexylamine	1.207	0	5.113	3.115	1.849	1.058	37.091	-0.574
Heptylamine	1.457	0	5.82	3.615	2.203	1.308	42.291	-0.615
Octylamine	1.707	0	6.527	4.115	2.556	1.558	47.491	-0.628
Nonylamine	1.957	0	7.234	4.615	2.91	1.808	52.691	-0.579
Decylamine	2.207	0	7.941	5.115	3.264	2.058	57.891	-0.628
Diethylamine	0.707	0	3.914	2.121	0.957	0.5	26.714	-0.55
Dipropylamine	1.207	0	5.328	3.121	1.75	0.854	37.114	-0.582
Diisopropylamine	0.943	0.816	5.655	2.887	2.476	0.667	37.11	-0.561
Dibutylamine	1.707	0	6.743	4.121	2.457	1.414	47.514	-0.598
Diisobutylamine	1.563	0.816	7.069	3.833	3.446	1.105	47.51	-0.547
Dipentylamine	2.207	0	8.157	5.121	3.164	1.914	57.914	-0.565
Piperidine	1.5	0	4.036	2.707	1.811	1.207	29.568	-0.556
Morpholine	1.5	0	3.737	2.284	1.362	0.846	26.848	-0.528
2-Methylpiperidine	1.894	0.289	4.906	3.128	2.351	1.557	34.766	-0.556
4-Methylpiperidine	1.894	0.289	4.906	3.101	2.433	1.628	34.766	-0.537
2-Ethylpiperidine	2.302	0.204	5.613	3.666	2.555	1.905	39.966	-0.561
Hexamethyleneimine	1.75	0	4.743	3.207	2.164	1.457	34.768	-0.521
Dicyclohexylamine	4.116	0.408	8.726	6.21	4.837	3.682	63.618	-0.637
Triethylamine	1.732	0.204	5.569	3.07	1.62	1.342	37.156	-0.341
Tripropylamine	2.091	0.204	7.69	4.57	2.842	1.62	52.756	-0.408
Tributylamine	2.898	0.204	9.811	6.07	3.902	2.484	68.356	-0.366
Dimethylcyclohexylamine	2.593	0.5	6.56	3.969	3.255	2.305	45.208	-0.35
Dimethylethylamine	0.816	0.408	4.154	1.918	1.396	0.632	26.756	-0.318
Diethylmethylamine	1.394	0.289	4.861	2.494	1.489	1.08	31.956	-0.337
Dimethylbutylamine	1.135	0.408	5.569	2.918	2.157	0.959	37.156	-0.316
Diisopropylethylamine	2.184	0.803	7.309	3.849	3.018	1.692	47.552	-0.437
1-Methylpiperidine	1.894	0.289	4.983	3.08	2.364	1.58	34.81	-0.382
1-Ethylpiperidine	2.302	0.204	5.69	3.656	2.495	1.896	40.01	-0.292

Table A 2: Correlation matrix for the toxicity and the 37 molecular descriptors, based on data for 36 aliphatic amines (N-Gast. = N-Gasteiger).

	log K_{ow} (est)	1/logLC$_{50}$	log K_{ow} (exp)	log K_{ow} (adj)	pK_a	N-Gast.	e_{HOMO}	e_{LUMO}	Diff	Hardness	EN	HOF	Dipol	D_{max}	D_{eff}	D_{min}	SASA	SAVOL	V^+
log K_{ow} (est.)	1																		
1/logLC$_{50}$	0.64	1																	
log K_{ow} (exp.)	0.99	0.67	1																
log K_{ow} (adj.)	0.99	0.70	0.99	1															
pK_a	0.34	0.23	0.34	0.31	1														
N-Gasteiger	0.39	0.09	0.39	0.37	0.42	1													
e_{HOMO}	0.20	-0.15	0.17	0.16	-0.02	0.79	1												
e_{LUMO}	-0.31	0.13	-0.26	-0.27	0.28	-0.53	-0.83	1											
Diff	-0.26	0.15	-0.22	-0.22	0.16	-0.68	-0.95	0.96	1										
Hardness	-0.27	0.15	-0.23	-0.23	0.16	-0.68	-0.95	0.96	1.00	1									
EN	0.20	0.02	0.18	0.20	-0.45	-0.36	-0.19	-0.39	-0.12	-0.11	1								
HOF	-0.78	-0.49	-0.77	-0.79	0.04	0.12	0.08	0.28	0.11	0.11	-0.61	1							
Dipol	-0.26	0.17	-0.22	-0.22	0.13	-0.61	-0.82	0.91	0.91	0.91	-0.25	0.14	1						
D_{max}	0.82	0.80	0.83	0.85	0.23	0.03	-0.11	-0.10	0.01	0.00	0.33	-0.82	-0.05	1					
D_{eff}	0.38	-0.32	0.34	0.32	-0.01	0.51	0.56	-0.62	-0.62	-0.61	0.17	-0.26	-0.65	-0.08	1				
D_{min}	0.29	0.01	0.29	0.29	0.00	0.48	0.57	-0.45	-0.53	-0.53	-0.14	-0.10	-0.32	-0.04	0.40	1			
SASA	0.96	0.61	0.95	0.95	0.20	0.26	0.17	-0.38	-0.28	-0.29	0.38	-0.90	-0.31	0.89	0.37	0.21	1		
SAVOL	0.97	0.60	0.96	0.96	0.19	0.30	0.20	-0.40	-0.32	-0.32	0.36	-0.89	-0.33	0.86	0.41	0.26	1.00	1	
V^+	-0.34	-0.31	-0.36	-0.35	-0.35	-0.17	-0.04	-0.01	0.01	0.01	0.10	0.24	0.08	-0.52	0.15	0.33	-0.38	-0.35	1
V^-	0.16	0.05	0.16	0.14	0.16	0.32	0.39	-0.32	-0.36	-0.37	-0.08	-0.06	-0.41	0.27	0.03	-0.25	0.22	0.20	-0.87
V^{tot}	-0.26	-0.19	-0.27	-0.25	-0.26	-0.25	-0.22	0.16	0.19	0.20	0.09	0.16	0.26	-0.41	0.06	0.30	-0.31	-0.28	0.97
Q^+_{max}	-0.03	0.08	-0.02	-0.01	0.15	-0.27	-0.32	0.29	0.31	0.32	0.02	0.01	0.42	-0.01	-0.32	0.28	-0.11	-0.09	0.50
Q^-_{max}	-0.03	-0.24	-0.04	-0.04	-0.30	0.59	0.77	-0.68	-0.75	-0.76	-0.07	0.15	-0.72	-0.21	0.51	0.30	0.02	0.04	-0.09
Q_{tot}	0.74	0.45	0.71	0.72	0.08	0.17	0.14	-0.39	-0.28	-0.28	0.43	-0.71	-0.23	0.58	0.31	0.46	0.72	0.75	0.26
Q_{av}	-0.54	-0.40	-0.56	-0.55	-0.19	-0.39	-0.27	0.18	0.23	0.24	0.13	0.33	0.23	-0.48	-0.22	-0.04	-0.54	-0.53	0.75
H^+_{max}	-0.07	0.17	-0.06	-0.07	0.33	-0.58	-0.70	0.67	0.72	0.72	-0.02	-0.05	0.72	0.12	-0.60	-0.27	-0.13	-0.14	0.04
MW	0.96	0.53	0.94	0.93	0.12	0.31	0.28	-0.46	-0.39	-0.39	0.35	-0.85	-0.37	0.77	0.47	0.34	0.96	0.97	-0.26
^0_C	0.96	0.54	0.95	0.95	0.14	0.35	0.28	-0.48	-0.40	-0.40	0.37	-0.87	-0.38	0.78	0.48	0.38	0.97	0.99	-0.24
^1_C	0.95	0.53	0.93	0.92	0.12	0.28	0.26	-0.44	-0.37	-0.37	0.33	-0.83	-0.35	0.76	0.45	0.30	0.94	0.96	-0.29
^2_C	0.85	0.38	0.82	0.80	0.05	0.38	0.38	-0.49	-0.45	-0.45	0.24	-0.67	-0.36	0.53	0.51	0.50	0.78	0.82	-0.04
$^3_C{}^p$	0.77	0.35	0.74	0.71	-0.01	0.36	0.42	-0.51	-0.48	-0.48	0.19	-0.54	-0.43	0.44	0.51	0.40	0.69	0.73	-0.12
$^3_C{}^c$	-0.04	-0.20	-0.05	-0.05	-0.08	0.38	0.33	-0.26	-0.31	-0.31	-0.06	0.13	-0.17	-0.33	0.29	0.56	-0.13	-0.08	0.52
$^0_C{}^v$	0.96	0.52	0.94	0.94	0.15	0.41	0.35	-0.52	-0.46	-0.46	0.34	-0.84	-0.43	0.76	0.51	0.41	0.96	0.98	-0.25
$^1_C{}^v$	0.97	0.55	0.95	0.94	0.16	0.32	0.27	-0.42	-0.36	-0.36	0.30	-0.82	-0.35	0.77	0.46	0.31	0.94	0.96	-0.29
$^2_C{}^v$	0.89	0.47	0.87	0.85	0.12	0.38	0.32	-0.40	-0.37	-0.37	0.18	-0.69	-0.29	0.59	0.46	0.47	0.81	0.85	-0.09
$^3_C{}^{v.}$	0.81	0.44	0.79	0.76	0.04	0.37	0.36	-0.43	-0.41	-0.41	0.16	-0.54	-0.38	0.51	0.45	0.35	0.71	0.75	-0.16
MOLVOL	0.97	0.56	0.96	0.96	0.16	0.34	0.27	-0.45	-0.38	-0.38	0.34	-0.86	-0.36	0.79	0.47	0.34	0.97	0.99	-0.28
N_ESP	-0.03	-0.25	-0.03	-0.03	-0.31	0.59	0.78	-0.69	-0.77	-0.77	-0.07	0.15	-0.73	-0.21	0.51	0.33	0.02	0.04	-0.07

Table A 2: (Continued)

	V^-	V^{tot}	Q^+_{max}	Q^-_{max}	Q_{tot}	Q_{av}	H^+_{max}	MW	^0C	^1C	^2C	$^3C^p$	$^3C^c$	$^0C^v$	$^1C^v$	$^2C^v$	$^3C^{v.}$	MOLVOL	N-ESP
V^-	1																		
V^{tot}	-0.97	1																	
Q^+_{max}	-0.65	0.59	1																
Q^-_{max}	0.43	-0.27	-0.69	1															
Q_{tot}	-0.34	0.31	0.43	-0.20	1														
Q_{av}	-0.67	0.74	0.61	-0.38	0.10	1													
H^+_{max}	-0.33	0.19	0.68	-0.93	0.10	0.38	1												
MW	0.16	-0.22	-0.07	0.07	0.77	-0.51	-0.15	1											
^0C	0.13	-0.19	-0.07	0.09	0.79	-0.49	-0.19	0.99	1										
^1C	0.20	-0.25	-0.09	0.05	0.73	-0.53	-0.13	0.99	0.96	1									
^2C	0.01	-0.02	0.01	0.11	0.76	-0.35	-0.14	0.91	0.89	0.90	1								
$^3C^p$	0.16	-0.15	-0.10	0.19	0.60	-0.47	-0.23	0.85	0.78	0.88	0.89	1							
$^3C^c$	-0.42	0.48	0.16	0.23	0.25	0.31	-0.18	-0.01	0.06	-0.09	0.31	0.01	1						
$^0C^v$	0.17	-0.22	-0.09	0.15	0.78	-0.51	-0.23	0.98	1.00	0.96	0.89	0.79	0.09	1					
$^1C^v$	0.19	-0.25	-0.07	0.04	0.74	-0.53	-0.13	0.99	0.97	1.00	0.90	0.88	-0.07	0.96	1				
$^2C^v$	0.01	-0.05	0.01	0.06	0.75	-0.41	-0.10	0.93	0.90	0.92	0.99	0.88	0.26	0.90	0.92	1			
$^3C^{v.}$	0.15	-0.16	-0.09	0.14	0.60	-0.49	-0.19	0.86	0.79	0.89	0.88	0.99	-0.02	0.80	0.89	0.89	1		
MOLVOL	0.16	-0.23	-0.08	0.07	0.77	-0.52	-0.16	1.00	0.99	0.98	0.89	0.81	0.00	0.99	0.99	0.91	0.83	1	
N_ESP	0.41	-0.25	-0.66	1.00	-0.18	-0.37	-0.93	0.08	0.10	0.06	0.12	0.20	0.25	0.16	0.05	0.07	0.15	0.08	1

Table B 1- B 36: Qualitative data (test conditions, lethal and sublethal endpoints)

Propylamine	**Control**		**651.3 [µmolL^{-1}]**		**857 [µmolL^{-1}]**		**1,101 [µmolL^{-1}]**		**1,422.4 [µmolL^{-1}]**		**1,861 [µmolL^{-1}]**	
O_2 [mgL^{-1}] (t_0)	8.12		8.18		7.90		8.07		7.99		8.09	
pH (t_0)	7.39		10.58		10.72		10.82		10.91		11.03	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	1	2	4	3	10	20	20
no heartbeat		0		0		0		0		3		0
sublethal effects												
no spontaneous movement	0		0		0		2		2		0	
tail not detached	0		0		0		0		0		0	
no blood circulation		0		0		0		0		9		0
lack of sacculus		0		0		0		0		0		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		0		5		0
sacculus with granulated otoliths		0		0		0		0		0		0
hypopigmentation		0		0		0		2		8		0
pericard oedema		0		1		1		2		3		0
yolk sack oedema		0		0		0		0		4		0

LC_{50} = 1,338.5 µmolL^{-1} (95% confidence: 1,200 – 1,492 µmolL^{-1})

Isopropylamine	**Control**		**881.4 [µmolL^{-1}]**		**1,233.3 [µmolL^{-1}]**		**1,727.3 [µmolL^{-1}]**		**2,415.8 [µmolL^{-1}]**		**3,383.5 [µmolL^{-1}]**	
O_2 [mgL^{-1}] (t_0)	8.5		7.92		7.81		7.56		7.62		7.45	
pH (t_0)	7.68		10.54		10.62		10.72		10.86		11.02	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	1	1	1	1	17	19
no heartbeat		0		0		0		1		4		0
sublethal effects												
no spontaneous movement	0		1		0		1		4		1	
tail not detached	0		0		0		0		0		0	
no blood circulation		0		1		2		1		4		1
lack of sacculus		0		0		0		0		0		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		0		6		1
sacculus with granulated otoliths		0		0		0		0		0		0
hypopigmentation		0		1		0		1		12		1
pericard oedema		0		0		1		1		1		0
yolk sack oedema		0		1		3		0		7		1

LC_{50} = 2,.531 µmolL^{-1} (95% confidence: 2,322 – 2,575 µmolL^{-1})

Butylamine	**Control**		**284.7 [µmolL^{-1}]**		**398.6 [µmolL^{-1}]**		**558 [µmolL^{-1}]**		**781.2 [µmolL^{-1}]**		**1,093.8 [µmolL^{-1}]**	
O_2 [mgL^{-1}] (t_0)	9.03		8.93		8.88		8.67		8.59		8.63	
pH (t_0)	7.58		10.26		10.46		10.57		10.77		10.91	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	1	5	5	7	7	20	20	20	20
no heartbeat		0		0		0		0		0		0
sublethal effects												
no spontaneous movement	0		0		0		0		0		0	
tail not detached	0		0		0		0		0		0	
no blood circulation		0		0		0		2		0		0
lack of sacculus		0		0		0		0		0		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		0		0		0
sacculus with granulated otoliths		0		0		0		1		0		0
hypopigmentation		0		0		0		2		0		0
pericard oedema		0		0		0		1		0		0
yolk sack oedema		0		0		0		1		0		0

LC_{50} = 491 µmolL^{-1} (95% confidence: 456 – 528 µmolL^{-1})

Isobutylamine	**Control**		**270 [µmolL^{-1}]**		**405.1 [µmolL^{-1}]**		**607.7 [µmolL^{-1}]**		**911.5 [µmolL^{-1}]**		**1367.2 [µmolL^{-1}]**	
O_2 [mgL^{-1}] (t_0)	8.47		8.35		8.25		8.01		8.00		7.94	
pH (t_0)	7.63		10.25		10.51		10.68		10.83		10.98	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	1	1	0	1	3	3
no heartbeat		0		0		0		1		3		9
sublethal effects												
no spontaneous movement	0		0		0		0		0		2	
tail not detached	0		0		0		0		0		1	
no blood circulation		0		0		3		2		4		11
lack of sacculus		0		0		0		0		0		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		0		0		7
sacculus with granulated otoliths		0		0		0		0		0		0
hypopigmentation		0		0		0		0		0		8
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		2		3		4		5		13

LC_{50} = 1,301 µmolL^{-1} (95% confidence: 1,150 – 1,472 µmolL^{-1})

sec-Butylamine	**Control**		**270 [µmolL^{-1}]**		**405.1 [µmolL^{-1}]**		**607.7 [µmolL^{-1}]**		**911.5 [µmolL^{-1}]**		**1367.2 [µmolL^{-1}]**	
O_2 [mgL^{-1}] (t_0)	8.47		8.35		8.25		8.01		8.00		7.94	
pH (t_0)	7.63		10.25		10.51		10.68		10.83		10.98	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	1	1	0	1	3	3
no heartbeat		0		0		0		1		3		9
sublethal effects												
no spontaneous movement	0		0		0		0		0		2	
tail not detached	0		0		0		0		0		1	
no blood circulation		0		0		3		2		4		11
lack of sacculus		0		0		0		0		0		0
sacculus with one otolith		0		0		0		0		0		1
sacculus without otoliths		0		0		0		0		0		7
sacculus with granulated otoliths		0		0		0		0		0		3
hypopigmentation		0		0		0		0		0		8
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		2		3		4		5		13

LC_{50} = 1,301 µmolL^{-1} (95% confidence: 1,150 – 1,472 µmolL^{-1})

Pentylamine	**Control**		**301.2 [µmolL^{-1}]**		**391.6 [µmolL^{-1}]**		**509.3 [µmolL^{-1}]**		**660.4 [µmolL^{-1}]**		**860.4 [µmolL^{-1}]**	
O_2 [mgL^{-1}] (t_0)	8.46		8.38		8.20		8.17		8.12		7.98	
pH (t_0)	7.36		10.31		10.45		10.62		10.72		10.86	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	7	11	13	14	16	16	20	20
no heartbeat		0		0		0		0		0		0
sublethal effects												
no spontaneous movement	0		0		1		0		3		0	
tail not detached	0		0		0		0		3		0	
no blood circulation		0		0		0		0		1		0
lack of sacculus		0		0		0		0		0		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		0		0		0
sacculus with granulated otoliths		0		0		0		0		1		0
hypopigmentation		0		1		2		0		0		0
pericard oedema		0		4		1		0		0		0
yolk sack oedema		0		1		1		0		1		0

LC_{50} = 354 µmolL^{-1} (95% confidence: 221.4 – 565 µmolL^{-1})

Isopentylamine	**Control**		**341.4 [µmolL^{-1}]**		**443.7 [µmolL^{-1}]**		**576.9 [µmolL^{-1}]**		**750 [µmolL^{-1}]**		**975.1 [µmolL^{-1}]**	
O_2 [mgL^{-1}] (t_0)	8.28		8.01		7.84		7.71		7.57		7.51	
pH (t_0)	7.62		10.37		10.48		10.63		10.70		10.82	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	1	1	1	1	2	2	10	15	20	20
no heartbeat		0		0		0		2		0		0
sublethal effects												
no spontaneous movement	0		0		0		1		5		0	
tail not detached	0		0		0		0		5		0	
no blood circulation		0		0		0		2		2		0
lack of sacculus		0		0		0		0		0		0
sacculus with one otolith		0		0		0		0		1		0
sacculus without otoliths		0		0		0		0		0		0
sacculus with granulated otoliths		0		0		0		1		2		0
hypopigmentation		0		0		0		1		1		0
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		0		0		3		3		0

LC_{50} = 678 µmolL^{-1} (95% confidence: 591.3 – 776.6 µmolL^{-1})

Cyclohexylamine	**Control**		**353 [µmolL^{-1}]**		**458.9 [µmolL^{-1}]**		**596.6 [µmolL^{-1}]**		**775.6 [µmolL^{-1}]**		**1008.3 [µmolL^{-1}]**	
O_2 [mgL^{-1}] (t_0)	8.98		8.92		8.88		8.79		8.52		8.77	
pH (t_0)	7.39		10.42		10.60		10.71		10.82		10.91	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	5	8	16	16	19	19
no heartbeat		0		0		2		0		0		0
sublethal effects												
no spontaneous movement	0		0		0		5		1		0	
tail not detached	0		0		0		1		0		1	
no blood circulation		0		0		2		2		1		0
lack of sacculus		0		0		0		0		0		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		1		2		0
sacculus with granulated otoliths		0		0		0		0		0		0
hypopigmentation		0		0		0		0		0		0
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		0		2		5		3		1

LC_{50} = 639 µmolL^{-1} (95% confidence: 584.2 – 697.9 µmolL^{-1})

Hexylamine	**Control**		**242.2 [µmolL^{-1}]**		**314.8 [µmolL^{-1}]**		**409.3 [µmolL^{-1}]**		**532.2 [µmolL^{-1}]**		**691.8 [µmolL^{-1}]**	
O_2 [mgL^{-1}] (t_0)	7.96		7.81		7.77		7.68		7.64		7.57	
pH (t_0)	7.56		10.16		10.33		10.48		10.61		10.72	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	1	1	1	1	3	10	16	17	20	20
no heartbeat		0		0		0		0		0		0
sublethal effects												
no spontaneous movement	0		0		0		7		3		0	
tail not detached	0		0		0		2		1		0	
"*Spina bifida*"	0		0		0		1		0		0	
no blood circulation		0		0		0		0		0		0
lack of sacculus		0		0		0		0		0		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		0		0		0
sacculus with granulated otoliths		0		0		0		0		0		0
hypopigmentation		0		0		0		1		1		0
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		0		2		1		1		0

LC_{50} = 418 µmolL^{-1} (95% confidence: 380.6 – 459.5 µmolL^{-1})

Heptylamine	**Control**		**151.9 [µmolL^{-1}]**		**197.5 [µmolL^{-1}]**		**256.7 [µmolL^{-1}]**		**333.8 [µmolL^{-1}]**		**434 [µmolL^{-1}]**	
O_2 [mgL^{-1}] (t_0)	9.08		8.94		8.88		8.79		8.66		8.56	
pH (t_0)	7.43		9.97		10.17		10.31		10.47		10.57	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	1	1	1	1	4	7	18	19	20	20
no heartbeat		0		0		0		3		0		0
sublethal effects												
no spontaneous movement	0		0		0		1		12		0	
tail not detached	0		0		0		1		0		0	
no blood circulation		0		0		0		8		1		0
lack of sacculus		0		0		0		0		0		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		0		1		0
sacculus with granulated otoliths		0		0		0		3		0		0
hypopigmentation		0		0		0		2		1		0
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		0		0		6		0		0

LC_{50} = 247 µmolL^{-1} (95% confidence: 228 – 268.3 µmolL^{-1})

Octylamine	**Control**		**162.5 [µmolL^{-1}]**		**211.3 [µmolL^{-1}]**		**274.7 [µmolL^{-1}]**		**357 [µmolL^{-1}]**		**464.2 [µmolL^{-1}]**	
O_2 [mgL^{-1}] (t_0)	7.89		7.80		7.66		7.75		7.75		7.74	
pH (t_0)	7.43		10.02		10.24		10.33		10.43		10.55	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	1	20	20	20	20	20	20
no heartbeat		0		1		10		0		0		0
sublethal effects												
no spontaneous movement	0		1		20		0		0		0	
tail not detached	0		1		0		0		0		0	
no blood circulation		0		1		15		0		0		0
lack of sacculus		0		0		0		0		0		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		0		0		0
sacculus with granulated otoliths		0		0		6		0		0		0
hypopigmentation		0		0		7		0		0		0
pericard oedema		0		0		1		0		0		0
yolk sack oedema		0		2		14		0		0		0

LC_{50} = 197 µmolL^{-1} (95% confidence: 186.6 – 207.2 µmolL^{-1})

Nonylamine	**Control**		**75.2 [µmolL^{-1}]**		**97.7 [µmolL^{-1}]**		**127 [µmolL^{-1}]**		**165.4 [µmolL^{-1}]**		**214.9 [µmolL^{-1}]**	
O_2 [mgL^{-1}] (t_0)	7.64		7.45		7.47		7.12		7.12		7.17	
pH (t_0)	7.69		9.81		9.96		10.12		10.27		10.41	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	1	4	9	17	8	20	20	20	20	20
no heartbeat		0		3		1		0		0		0
sublethal effects												
no spontaneous movement	0		7		11		11		0		0	
tail not detached	0		2		3		2		0		0	
no blood circulation		0		11		3		0		0		0
lack of sacculus		0		0		0		0		0		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		1		0		0		0		0
sacculus with granulated otoliths		0		3		0		0		0		0
hypopigmentation		0		14		3		0		0		0
pericard oedema		0		3		0		0		0		0
yolk sack oedema		0		9		3		0		0		0

LC_{50} = 80 µmolL^{-1} (95% confidence: 75.5 – 85.4 µmolL^{-1})

Decylamine	**Control**		**13.2 [µmolL^{-1}]**		**18.6 [µmolL^{-1}]**		**25.9 [µmolL^{-1}]**		**36.3 [µmolL^{-1}]**		**50.9 [µmolL^{-1}]**	
O_2 [mgL^{-1}] (t_0)	7.72		7.98		7.91		7.89		7.56		7.42	
pH (t_0)	7.52		9.32		9.38		9.47		9.70		9.96	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	1	0	1	0	4	5	20	11	20
no heartbeat		0		1		5		13		0		0
sublethal effects												
no spontaneous movement	0		14		20		20		15		9	
tail not detached	0		0		0		0		0		0	
no blood circulation		0		4		10		14		0		0
lack of sacculus		0		0		0		0		0		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		0		0		0
sacculus with granulated otoliths		0		5		9		0		0		0
hypopigmentation		0		19		17		15		0		0
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		0		0		3		0		0

LC_{50} = 20 µmolL^{-1} (95% confidence: 17.9 – 22.3 µmolL^{-1})

Diethylamine	**Control**		**526.4 [µmolL^{-1}]**		**685 [µmolL^{-1}]**		**890.1 [µmolL^{-1}]**		**1,156.7 [µmolL^{-1}]**		**1,504 [µmolL^{-1}]**	
O_2 [mgL^{-1}] (t_0)	8.66		8.41		8.15		8.15		8.28		8.31	
pH (t_0)	7.28		10.53		10.69		10.84		10.97		11.07	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	0	0	1	1	12	18
no heartbeat		0		0		0		0		1		0
sublethal effects												
no spontaneous movement	0		0		0		0		0		5	
tail not detached	0		0		0		0		0		0	
no blood circulation		0		0		0		0		6		1
lack of sacculus		0		0		0		0		3		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		0		2		0
sacculus with granulated otoliths		0		0		1		0		3		0
hypopigmentation		0		0		0		0		11		1
pericard oedema		0		0		0		2		8		1
yolk sack oedema		0		0		0		0		3		0

LC_{50} = 1,275 µmolL^{-1} (95% confidence: 1,211 – 1,344 µmolL^{-1})

Dipropylamine	**Control**		**207.5 [µmolL^{-1}]**		**269.9 [µmolL^{-1}]**		**350.8 [µmolL^{-1}]**		**456.1 [µmolL^{-1}]**		**592.9 [µmolL^{-1}]**	
O_2 [mgL^{-1}] (t_0)	7.64		7.82		7.74		7.87		7.76		7.75	
pH (t_0)	7.39		10.44		10.64		10.78		10.93		11.05	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	17	17	20	20	20	20
no heartbeat		0		0		3		0		0		0
sublethal effects												
no spontaneous movement	0		7		15		1		0		0	
tail not detached	0		2		9		0		0		0	
no blood circulation		0		0		5		0		0		0
lack of sacculus		0		0		1		0		0		0
sacculus with one otolith		0		0		1		0		0		0
sacculus without otoliths		0		0		0		0		0		0
sacculus with granulated otoliths		0		0		2		1		0		0
hypopigmentation		0		0		5		1		0		0
pericard oedema		0		0		4		0		0		0
yolk sack oedema		0		0		1		0		0		0

LC_{50} = 308 µmolL^{-1} (95% confidence: 291 – 325 µmolL^{-1})

Diisopropylamine	**Control**		**415.2 [µmolL^{-1}]**		**539.6 [µmolL^{-1}]**		**701.7 [µmolL^{-1}]**		**912.2 [µmolL^{-1}]**		**1,186 [µmolL^{-1}]**	
O_2 [mgL^{-1}] (t_0)	7.61		7.55		7.39		7.33		7.28		7.41	
pH (t_0)	7.52		10.08		10.22		10.37		10.47		10.56	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	0	0	4	12	18	20
no heartbeat		0		0		0		0		1		0
sublethal effects												
no spontaneous movement	0		0		9		20		15		1	
tail not detached	0		0		0		0		0		1	
no blood circulation		0		0		0		0		1		0
lack of sacculus		0		0		0		0		2		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		4		1		0
sacculus with granulated otoliths		0		0		0		0		0		0
hypopigmentation		0		0		0		0		4		0
pericard oedema		0		1		2		1		3		0
yolk sack oedema		0		0		0		3		1		0

LC_{50} = 904 µmolL^{-1} (95% confidence: 867 – 943 µmolL^{-1})

Dibutylamine	**Control**		**216.7 [µmolL^{-1}]**		**281.7 [µmolL^{-1}]**		**366.3 [µmolL^{-1}]**		**476.1 [µmolL^{-1}]**		**619 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	8.78		8.54		8.57		8.63		8.55		8.69	
pH (t_0)	7.76		10.20		10.37		10.51		10.62		10.77	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	4	5	15	16	19	19	20	20
no heartbeat		0		0		1		0		0		0
sublethal effects												
no spontaneous movement	0		12		8		2		0		0	
tail not detached	0		4		8		2		0		0	
"*Spina bifida*"	0		1		5		3		0		0	
no blood circulation		0		0		4		2		1		0
lack of sacculus		0		0		0		0		0		0
sacculus with one otolith		0		0		0		1		1		0
sacculus without otoliths		0		0		0		0		0		0
sacculus with granulated otoliths		0		1		0		0		0		0
hypopigmentation		0		1		4		0		0		0
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		2		5		3		0		0

LC_{50} = 313 µmolL^{-1} (95% confidence: 283 – 345 µmolL^{-1})

Diisobutylamine	**Control**		**270.8 [µmolL^{-1}]**		**352.2 [µmolL^{-1}]**		**457.8 [µmolL^{-1}]**		**595.1 [µmolL^{-1}]**		**773.7 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	9.12		8.67		8.52		8.13		8.18		8.03	
pH (t_0)	7.48		10.63		10.73		10.83		10.94		11.05	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	3	4	18	18	20	20	20	20
no heartbeat		0		1		4		0		0		0
sublethal effects												
no spontaneous movement	0		8		9		1		0		0	
tail not detached	0		2		2		0		0		0	
"*Spina bifida*"	0		0		3		0		0		0	
no blood circulation		0		1		10		0		0		0
lack of sacculus		0		0		0		0		0		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		0		0		0
sacculus with granulated otoliths		0		0		4		1		0		0
hypopigmentation		0		0		1		0		0		0
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		4		7		0		0		0

LC_{50} = 365 µmolL^{-1} (95% confidence: 340 – 393 µmolL^{-1})

Dipentylamine	**Control**		**178.1 [µmolL^{-1}]**		**231.5 [µmolL^{-1}]**		**301 [µmolL^{-1}]**		**391 [µmolL^{-1}]**		**508.6 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	7.5		7.46		7.49		7.35		7.32		7.26	
pH (t_0)	7.53		10.27		10.40		10.57		10.69		10.84	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	2	2	0	2	1	3	18	19	20	20
no heartbeat		0		0		3		6		0		0
sublethal effects												
no spontaneous movement	0		0		15		2		2		0	
tail not detached	0		0		1		3		2		0	
no blood circulation		0		0		3		11		1		0
lack of sacculus		0		0		0		0		0		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		0		0		0
sacculus with granulated otoliths		0		0		0		1		0		0
hypopigmentation		0		0		1		2		1		0
pericard oedema		0		0		0		1		0		0
yolk sack oedema		0		4		7		14		0		0

LC_{50} = 272 µmolL^{-1} (95% confidence: 248 – 299 µmolL^{-1})

Piperidine	**Control**		**575.7 [µmolL^{-1}]**		**748.3 [µmolL^{-1}]**		**972.9 [µmolL^{-1}]**		**1,265 [µmolL^{-1}]**		**1,644 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	7.54		7.26		7.32		7.13		7.10		7.19	
pH (t_0)	7.80		10.36		10.49		10.60		10.71		10.82	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	0	0	2	2	20	20
no heartbeat		0		0		0		0		1		0
sublethal effects												
no spontaneous movement	0		0		0		0		20		0	
tail not detached	0		0		0		0		0		0	
no blood circulation		0		0		0		0		1		0
lack of sacculus		0		0		0		0		1		0
sacculus with one otolith		0		0		0		0		3		0
sacculus without otoliths		0		0		0		0		0		0
sacculus with granulated otoliths		0		0		0		4		4		0
hypopigmentation		0		0		0		1		1		0
pericard oedema		0		0		0		0		1		0
yolk sack oedema		0		0		0		0		0		0

LC_{50} = 1,297 µmolL^{-1} (95% confidence: 1,226 – 1,370 µmolL^{-1})

Morpholine	**Control**		**2,869.6 [µmolL^{-1}]**		**4,591.4 [µmolL^{-1}]**		**7,346.2 [µmolL^{-1}]**		**11,754 [µmolL^{-1}]**		**18,806 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	7.67		7.58		7.47		7.51		7.26		7.49	
pH (t_0)	7.40		9.79		9.97		10.08		10.19		10.29	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	2	1	2	1	2	1	20	19	20
no heartbeat		0		0		3		9		0		0
sublethal effects												
no spontaneous movement	0		0		0		0		0		0	
tail not detached	0		0		0		0		0		0	
no blood circulation		0		0		3		9		0		0
lack of sacculus		0		0		0		4		0		0
sacculus with one otolith		0		0		1		5		0		0
sacculus without otoliths		0		0		0		0		0		0
sacculus with granulated otoliths		0		0		5		1		0		0
hypopigmentation		0		5		17		17		0		0
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		5		10		7		0		0

LC_{50} = 6,901 µmolL^{-1} (95% confidence: 5,042 – 9,446 µmolL^{-1})

2-Methylpiperidine	**Control**		**529.5 [µmolL^{-1}]**		**688.3 [µmolL^{-1}]**		**894.9 [µmolL^{-1}]**		**1,163.3 [µmolL^{-1}]**		**1,512.4 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	8.75		8.77		8.79		8.78		8.96		8.97	
pH (t_0)	7.33		10.58		10.74		10.86		10.96		11.09	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	2	2	3	10	20	20
no heartbeat		0		0		0		2		4		0
sublethal effects												
no spontaneous movement	0		1		5		17		16		0	
tail not detached	0		1		0		0		2		0	
no blood circulation		0		0		0		12		5		0
lack of sacculus		0		0		0		1		4		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		6		1		0
sacculus with granulated otoliths		0		0		0		0		0		0
hypopigmentation		0		0		0		7		4		0
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		0		3		14		6		0

LC_{50} = 1,032 µmolL^{-1} (95% confidence: 979 – 1,088 µmolL^{-1})

4-Methylpiperidine	**Control**		**423.6 [µmolL^{-1}]**		**550.7 [µmolL^{-1}]**		**715.9 [µmolL^{-1}]**		**930.7 [µmolL^{-1}]**		**1,210 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	8.86		8.46		8.52		8.54		8.31		8.22	
pH (t_0)	7.32		10.47		10.74		10.81		10.95		11.10	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	0	0	0	3	10	18
no heartbeat		0		0		0		1		6		1
sublethal effects												
no spontaneous movement	0		0		0		5		11		3	
tail not detached	0		0		0		1		1		3	
no blood circulation		0		0		0		1		8		1
lack of sacculus		0		0		0		1		6		1
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		0		2		0
sacculus with granulated otoliths		0		0		0		0		0		0
hypopigmentation		0		0		0		1		8		1
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		0		0		1		7		1

LC_{50} = 937 µmolL^{-1} (95% confidence: 876 – 1,002 µmolL^{-1})

Hexamethyleneimine	**Control**		**529.5 [µmolL^{-1}]**		**688.3 [µmolL^{-1}]**		**894.9 [µmolL^{-1}]**		**1,163.3 [µmolL^{-1}]**		**1,512.4 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	9.2		8.89		8.60		8.58		8.92		8.83	
pH (t_0)	7.21		10.63		10.76		10.89		11.02		11.19	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	1	1	1	5	16	17
no heartbeat		0		0		0		1		7		0
sublethal effects												
no spontaneous movement	0		0		0		0		13		0	
tail not detached	0		0		0		0		3		0	
no blood circulation		0		0		0		2		10		0
lack of sacculus		0		0		0		1		10		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		0		0		0
sacculus with granulated otoliths		0		0		0		0		0		0
hypopigmentation		0		0		0		1		11		0
pericard oedema		0		0		0		2		0		0
yolk sack oedema		0		0		0		3		10		1

LC_{50} = 1,163 µmolL^{-1} (95% confidence: 1,068 – 1,265 µmolL^{-1})

2-Ethylpiperidine	**Control**		**309.3 [µmolL^{-1}]**		**402 [µmolL^{-1}]**		**522.7 [µmolL^{-1}]**		**679.5 [µmolL^{-1}]**		**883.4 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	8.57		8.61		8.55		8.61		8.51		8.56	
pH (t_0)	7.70		10.37		10.55		10.71		10.84		10.94	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	0	0	0	0	5	9
no heartbeat		0		0		0		0		1		5
sublethal effects												
no spontaneous movement	0		0		16		20		18		10	
tail not detached	0		0		5		8		8		4	
no blood circulation		0		0		0		0		5		6
lack of sacculus		0		0		0		0		1		3
sacculus with one otolith		0		0		0		0		2		0
sacculus without otoliths		0		0		0		0		0		3
sacculus with granulated otoliths		0		0		0		0		1		0
hypopigmentation		0		0		0		0		4		6
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		0		0		0		12		7

LC_{50} = 830 µmolL^{-1} (95% confidence: 781 – 881 µmolL^{-1})

Dicyclohexylamine	**Control**		**108.9 [µmolL^{-1}]**		**163.4 [µmolL^{-1}]**		**245.1 [µmolL^{-1}]**		**367.7 [µmolL^{-1}]**		**551.5 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	8.7		8.41		8.21		8.03		7.98		7.87	
pH (t_0)	7.56		10.17		10.43		10.69		10.89		11.12	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	1	1	2	7	16	17	20	20	20	20
no heartbeat		0		0		4		0		0		0
sublethal effects												
no spontaneous movement	0		2		10		2		0		0	
tail not detached	0		0		1		2		0		0	
no blood circulation		0		0		7		1		0		0
lack of sacculus		0		0		1		0		0		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		0		0		0
sacculus with granulated otoliths		0		0		1		0		0		0
hypopigmentation		0		0		7		0		0		0
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		0		4		1		0		0

LC_{50} = 172 µmolL^{-1} (95% confidence: 152 – 193 µmolL^{-1})

N,N- Dimethylethylamine	**Control**		**405.2 [µmolL^{-1}]**		**607.7 [µmolL^{-1}]**		**911.7 [µmolL^{-1}]**		**1,367 [µmolL^{-1}]**		**2,051 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	8.6		8.5		8.66		8.73		8.70		8.62	
pH (t_0)	7.4		9.88		10.11		10.33		10.48		10.61	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	0	1	14	14	20	20
no heartbeat		0		0		0		2		1		0
sublethal effects												
no spontaneous movement	0		0		0		0		1		0	
tail not detached	0		0		0		0		1		0	
no blood circulation		0		0		0		10		2		0
lack of sacculus		0		0		0		0		1		0
sacculus with one otolith		0		0		0		1		0		0
sacculus without otoliths		0		0		0		1		1		0
sacculus with granulated otoliths		0		0		0		0		0		0
hypopigmentation		0		0		0		8		2		0
pericard oedema		0		0		0		1		0		0
yolk sack oedema		0		0		0		8		1		0

LC_{50} = 1,133 µmolL^{-1} (95% confidence: 1,040 – 1,235 µmolL^{-1})

N,N-Diethylmethylamine	**Control**		**339.9 [µmolL^{-1}]**		**509.9 [µmolL^{-1}]**		**765 [µmolL^{-1}]**		**1,147.4 [µmolL^{-1}]**		**1,721.2 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	8.54		8.57		8.44		8.48		8.44		8.57	
pH (t_0)	7.38		10.03		10.26		10.47		10.63		10.78	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	1	1	2	7	17	17	20	20
no heartbeat		0		0		0		4		0		0
sublethal effects												
no spontaneous movement	0		0		0		0		0		0	
tail not detached	0		0		0		0		0		0	
no blood circulation		0		0		1		9		0		0
lack of sacculus		0		0		0		4		1		0
sacculus with one otolith		0		0		0		1		0		0
sacculus without otoliths		0		0		0		5		0		0
sacculus with granulated otoliths		0		0		0		0		0		0
hypopigmentation		0		0		0		10		0		0
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		1		2		11		0		0

LC_{50} = 803 µmolL^{-1} (95% confidence: 714 – 903 µmolL^{-1})

N,N-Dimethylbutylamine	**Control**		**195.2 [µmolL^{-1}]**		**292.8 [µmolL^{-1}]**		**439.2 [µmolL^{-1}]**		**658.9 [µmolL^{-1}]**		**988.2 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	8.43		8.35		8.49		8.64		8.48		8.37	
pH (t_0)	7.34		9.76		10.02		10.25		10.35		10.51	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	1	2	18	19	20	20
no heartbeat		0		0		0		2		0		0
sublethal effects												
no spontaneous movement	0		0		12		13		0		0	
tail not detached	0		0		0		7		0		0	
"*Spina bifida*"	0		0		0		2		1		0	
no blood circulation		0		0		0		2		0		0
lack of sacculus		0		0		0		0		1		0
sacculus with one otolith		0		0		0		1		0		0
sacculus without otoliths		0		0		0		0		0		0
sacculus with granulated otoliths		0		0		0		0		0		0
hypopigmentation		0		0		0		0		0		0
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		0		0		3		0		0

LC_{50} = 504 µmolL^{-1} (95% confidence: 461 – 551 µmolL^{-1})

N,N-Dimethylcyclo-hexylamine	**Control**		**173.9 [µmolL^{-1}]**		**243.4 [µmolL^{-1}]**		**340.8 [µmolL^{-1}]**		**477.1 [µmolL^{-1}]**		**668.1 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	7.89		7.54		7.45		7.35		7.16		7.06	
pH (t_0)	7.40		9.84		10.07		10.22		10.42		10.66	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	0	0	14	15	20	20
no heartbeat		0		0		0		1		5		0
sublethal effects												
no spontaneous movement	0		0		0		0		0		0	
tail not detached	0		0		0		0		0		0	
no blood circulation		0		0		0		1		5		0
lack of sacculus		0		0		0		0		1		0
sacculus with one otolith		0		0		0		1		0		0
sacculus without otoliths		0		0		0		1		0		0
sacculus with granulated otoliths		0		0		0		0		0		0
hypopigmentation		0		0		0		1		3		0
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		0		0		0		1		0

LC_{50} = 417 µmolL^{-1} (95% confidence: 388 – 448 µmolL^{-1})

N,N-Diisopropyl-ethylamine	**Control**		**446.8 [µmolL^{-1}]**		**581.5 [µmolL^{-1}]**		**755.8 [µmolL^{-1}]**		**982.7 [µmolL^{-1}]**		**1,277.7 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	8.59		8.54		8.68		8.70		8.67		8.66	
pH (t_0)	7.34		10.57		10.69		10.81		10.96		11.08	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	0	0	9	15	19	19
no heartbeat		0		0		2		6		3		0
sublethal effects												
no spontaneous movement	0		13		14		20		9		1	
tail not detached	0		0		0		0		0		1	
no blood circulation		0		0		5		11		4		0
lack of sacculus		0		0		0		3		3		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		0		0		0
sacculus with granulated otoliths		0		0		0		0		0		0
hypopigmentation		0		0		2		8		3		0
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		0		5		12		4		1

LC_{50} = 809 µmolL^{-1} (95% confidence: 738 – 875 µmolL^{-1})

1-Methylpiperidine	**Control**		**275.3 [µmolL^{-1}]**		**357.9 [µmolL^{-1}]**		**465.3 [µmolL^{-1}]**		**605 [µmolL^{-1}]**		**786.4 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	8.75		8.67		8.61		8.56		8.78		8.62	
pH (t_0)	7.43		10.12		10.21		10.31		10.41		10.51	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	0	0	0	0	15	16
no heartbeat		0		0		0		0		4		1
sublethal effects												
no spontaneous movement	0		0		0		0		19		2	
tail not detached	0		0		0		0		0		2	
no blood circulation		0		0		0		0		10		2
lack of sacculus		0		0		0		0		1		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		0		4		0
sacculus with granulated otoliths		0		0		0		0		0		0
hypopigmentation		0		0		0		0		5		2
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		0		0		0		10		1

LC_{50} = 689 µmolL^{-1} (95% confidence: 651 – 731 µmolL^{-1})

1-Ethylpiperidine	**Control**		**309.3 [µmolL^{-1}]**		**402 [µmolL^{-1}]**		**522.7 [µmolL^{-1}]**		**679.5 [µmolL^{-1}]**		**883.4 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	8.89		8.90		8.84		8.89		8.94		8.95	
pH (t_0)	7.77		10.46		10.53		10.60		10.69		10.78	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	1	1	12	13	17	17
no heartbeat		0		0		0		4		0		1
sublethal effects												
no spontaneous movement	0		8		20		16		5		2	
tail not detached	0		1		0		2		1		2	
no blood circulation		0		0		0		6		2		2
lack of sacculus		0		0		0		0		0		0
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		2		0		0
sacculus with granulated otoliths		0		0		0		2		1		1
hypopigmentation		0		0		0		6		1		2
pericard oedema		0		0		0		0		0		0
yolk sack oedema		0		0		2		8		3		3

LC_{50} = 630 µmolL^{-1} (95% confidence: 564 – 703 µmolL^{-1})

Triethylamine	**Control**		**135.7 [µmolL^{-1}]**		**217.1 [µmolL^{-1}]**		**347.4 [µmolL^{-1}]**		**555.9 [µmolL^{-1}]**		**889.4 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	9.02		8.65		8.98		8.59		8.49		8.43	
pH (t_0)	7.45		9.95		10.25		10.49		10.68		10.86	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	0	0	0	5	18	18
no heartbeat		0		0		0		0		1		1
sublethal effects												
no spontaneous movement	0		0		0		0		10		1	
tail not detached	0		0		0		0		8		0	
no blood circulation		0		0		0		0		4		0
lack of sacculus		0		0		0		0		2		0
sacculus with one otolith		0		0		0		0		1		1
sacculus without otoliths		0		0		0		0		2		0
sacculus with granulated otoliths		0		2		1		0		2		1
hypopigmentation		0		1		0		0		5		1
pericard oedema		0		0		1		1		2		0
yolk sack oedema		0		0		0		0		2		1

LC_{50} = 598 µmolL^{-1} (95% confidence: 477 – 749 µmolL^{-1})

Tripropylamine	**Control**		**413.6 [µmolL^{-1}]**		**620.5 [µmolL^{-1}]**		**930.6 [µmolL^{-1}]**		**1,396 [µmolL^{-1}]**		**2,094 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)	9.23		9.08		9.02		8.89		8.82		8.76	
pH (t_0)	7.56		10.30		10.37		10.55		10.69		10.97	
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	1	1	3	0	5	19	19
no heartbeat		0		0		0		0		3		0
sublethal effects												
no spontaneous movement	0		0		1		8		14		1	
tail not detached	0		0		0		0		1		1	
no blood circulation		0		0		0		0		3		1
lack of sacculus		0		0		0		0		1		1
sacculus with one otolith		0		0		0		0		0		0
sacculus without otoliths		0		0		0		0		0		0
sacculus with granulated otoliths		0		0		0		0		1		0
hypopigmentation		0		0		0		0		2		1
pericard oedema		0		3		1		1		3		0
yolk sack oedema		0		1		1		0		1		0

LC_{50} = 1,318 µmolL^{-1} (95% confidence: 1,165 – 1,490 µmolL^{-1})

Tributylamine	**Control**		**67.4 [µmolL^{-1}]**		**134.9 [µmolL^{-1}]**		**269.7 [µmolL^{-1}]**		**539.5 [µmolL^{-1}]**		**1,079 [µmolL^{-}]**	
O_2 [mgL^{-1}] (t_0)												
pH (t_0)												
number of inividuals	20		20		20		20		20		20	
lethal effects	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**
coagulated	0	0	0	0	0	0	0	0	1	1	3	3
no heartbeat		0		0		0		0		0		0
sublethal effects												
no spontaneous movement	0		0		0		0		0		0	
tail not detached	0		0		0		0		0		0	
no blood circulation		0		0		0		0		0		0
lack of sacculus		0		0		0		0		0		0
sacculus with one otolith		0		0		0		0		0		1
sacculus without otoliths		0		0		0		0		0		0
sacculus with granulated otoliths		0		0		0		0		0		0
hypopigmentation		0		0		0		0		0		1
pericard oedema		0		0		0		0		0		1
yolk sack oedema		0		0		0		0		0		1

LC_{50} = 1,625 µmolL^{-1} (95% confidence: 869 – 3,038 µmolL^{-1})

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