Effects of Exposure to N-phenyl-α-naphthylamine, Octyl-phenyl-α-naphthylamine, and Dioctyldiphenylamine on the Development of Frog Embryos.

Gerald Greenhouse
Departments of Anatomy and Developmental and Cell Biology
University of California at Irvine
Irvine, Cal. 92717

This project was initiated as part of a larger study on the effects of environmental pollution resulting from United States Air Force operations. N-phenyl-α-naphthylamine, octyl-phenyl-α-naphthylamine, and dioctyldiphenylamine were designated as potential environmental pollutants by the United States Air Force. Previous work has shown that neither octyl-phenyl-α-naphthylamine nor dioctyldiphenylamine are toxic to frog larvae, whereas N-phenyl-α-naphthylamine is highly toxic to larvae (GREENHOUSE, 1976a). The objective of the experiments described below was to ascertain whether these compounds are teratogens.

MATERIAL AND METHODS

N-phenyl-α-naphthylamine, octyl-phenyl-α-naphthylamine, and dioctyldiphenylamine were supplied by the Toxic Hazards Division of the Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Dayton, Ohio.

Adult Rana pipiens and Xenopus laevis frogs were obtained and maintained as previously described (GREENHOUSE, 1976a,b). Fertilized eggs were obtained and cultured using standard laboratory procedures (RUGH, 1962; BROWN, 1970). R. pipiens embryos were staged according to SHUMWAY (1940), X. laevis embryos according to NIEUWKOOP and FABER (1956).

Chemically dejellied embryos (3% cysteine, 0.1% papain, pH 8.0) were exposed to the above compounds as follows. Batches of 50-100 embryos at the desired stage were placed in 19 cm inner diameter glass dishes containing one liter of an aqueous suspension or solution of the chemical to be tested. At intervals, embryos were removed from the contaminated solutions and placed in uncontaminated aged tap water. Only clutches of eggs which were at least 95% fertile were used, and each experimental group was paired with control eggs obtained from the same pair of mated frogs.

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The three organic amines used in these studies are relatively insoluble in water. Solutions were prepared by adding the dry amine to water and agitating the suspension on a reciprocating shaker for various periods of time. The suspension was then filtered and an aliquot of the filtrate extracted with hexane. The amount of amine in solution was then determined spectrophotometrically. The amount of amine in solution varied with the time on the shaker and the temperature. The relative solubility of N-phenyl-α-naphthylamine > octyl-phenyl-α-naphthylamine > dioctyldiphenylamine.

In some instances the embryos were exposed to suspensions of the compounds rather than filtered solutions.

Embryos and larvae were scored as normal or abnormal by a combination of macroscopic and microscopic observations. Animals were first examined using a dissecting microscope. Representative normal and malformed animals were then serially sectioned at 7-10μm and examined with a compound microscope.

Standard histological procedures were used in the preparation of embryological material for microscopic analysis. In order to facilitate the sectioning of yolk laden tissues, fixation was in Smith's fluid (RUGH, 1962) or Bouin's fluid diluted 1:1 with dioxane (PUCKETT, 1937).

RESULTS AND DISCUSSION

Exposure of frog embryos to octyl-phenyl-α-naphthylamine or dioctyldiphenylamine had no deleterious effects upon development (tables 1 & 2). Exposure of embryos to N-phenyl-α-naphthylamine resulted in teratogenesis and increased mortality (tables 1 & 2).

TABLE I

EFFECT OF EXPOSURE TO SUSPENSIONS OF ORGANIC AMINES ON DEVELOPMENT OF RANA PIPIENS EMBRYOS

Compound	Conc. mg/l	No. Emb. Exp.	No. Emb. Malf.
N-phenyl-α-naphthylamine	20	100	100
	200	100	100
Octyl-phenyl-α-naphthylamine	20	100	0
	200	100	0
Dioctyldiphenylamine	20	100	0
	200	100	0
Control		100	0

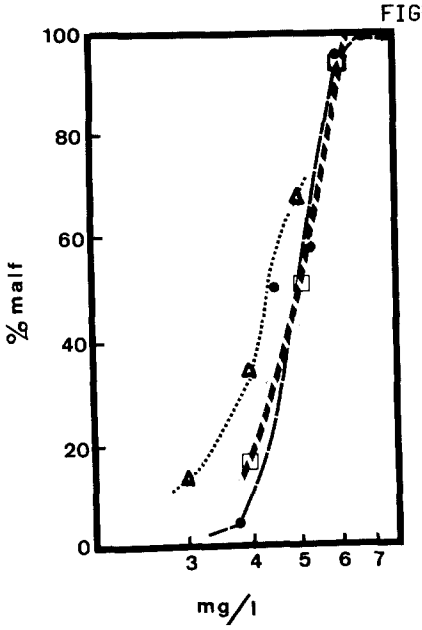
TABLE 2

EFFECT OF EXPOSURE TO SOLUTIONS OF ORGANIC AMINES ON DEVELOPMENT OF XENOPUS LAEVIS EMBRYOS

Compound	Conc. mg/l	No. Emb. Exp.	No. Emb. Malf.	% Malf.
Controls	0.0	175	2	3.5
N-phenyl-α-naphthylamine	6	176	167	94.8
Octylphenyl-α-naphthylamine	1 (saturated)	100	8	8

Although the relative sensitivity of the eggs to N-phenyl-α-naphthylamine varied from clutch to clutch, this compound was teratogenic to all clutches of eggs tested. Figure 1 shows the log dose-response curves for three separate experiments. From this data it is estimated that the ED_{50} is between 4 and 5 mg/l.

FIGURE 1

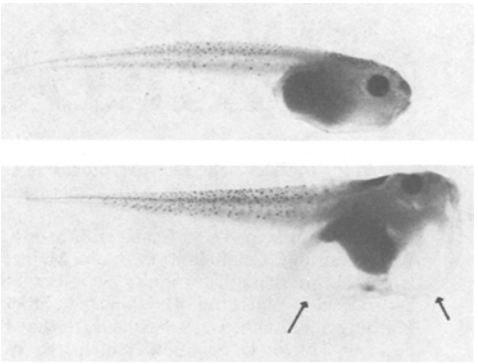


Log-dose % teratogenicity of N-phenyl-α-naphthylamine to frog embryos.

All known teratogens have a "critical period" of development during which they exert their toxic effects. Exposure to the compound at other developmental stages may be lethal but rarely leads to congenital defects. N-phenyl-α-naphthylamine conforms to this pattern. Embryos exposed during neurulation become malformed. Exposure of earlier or later stages may be toxic but is not teratogenic.

The syndrome of malformations induced by exposure of frog neuralae to N-phenyl-α-naphthylamine typically includes shortening of the trunk and intestinal tract and edema (fig. 2).

FIGURE 2



Teratogenic effect of N-phenyl-α-naphthylamine in X. laevis. Above-control larvae. Below-larva exposed to N-phenyl-α-naphthylamine. Note the edematous gut and pericardial regions (arrows).

Neither octyl-phenyl-α-naphthylamine nor dioctyldiphenylamine was found to be teratogenic or otherwise toxic to frog embryos. This is probably due to the insolubility of the latter and near insolubility of the former compound in aqueous media. Should these compounds enter aquatic systems accompanied by detergents or other emulsifiers their toxicity would have to be reevaluated.

The significance of these studies with regard to the potential danger of N-phenyl-α-naphthylamine to human embryos is uncertain. My data indicate that embryos in direct contact with this compound are at risk. Therefore, in the absence of contradictory evidence, N-phenyl-α-naphthylamine should be considered a potential teratogen. However, there are as yet no studies of the effects of these compounds on pregnant mammals or their offspring, nor is it known whether N-phenyl-α-naphthylamine or its metabolites can cross the placenta.

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