Toxicity of N-phenyl-α-naphthylamine and hydrazine to *Xenopus laevis* Embryos and Larvae

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A study of the effects of environmental pollution resulting from United States Air Force operations indicated that N-phenyl-α-naphthylamine, hydrazine, methylhydrazine, and dimethylhydrazine are highly toxic to embryos and larvae of two species of frog (GREENHOUSE, 1976a,b). Subsequent studies demonstrated that both N-phenyl-α-naphthylamine (GREENHOUSE, 1976c) and the hydrazines (GREENHOUSE, 1975, 1976d) are teratogens. The syndrome of malformations caused by exposure of embryos to these compounds and the stages of development during which embryos are susceptible to teratogenesis were also determined.

The data presented below establishes the LD50 of N-phenyl-α-naphthylamine for Xenopus laevis larvae and the ED50 (teratogenic) of N-phenyl-α-naphthylamine, hydrazine, and 1,1-dimethylhydrazine for X. laevis embryos.

MATERIALS and METHODS

N-phenyl-α-naphthylamine was supplied by the Aerospace Medical Research Laboratory, Dayton, Ohio. Hydrazine was purchased from Sigma Chemical Co., and 1,1-dimethylhydrazine was purchased from Research Organic/Inorganic Chemical Corp.

Adult X. laevis were captured in drainage ditches in Costa Mesa, California, or purchased from the South African Snake Farm, Fish Hoek, Cape Province, South Africa. Adult frogs were maintained in glass aquaria and fed Purina Trout Chow twice weekly.

Fertilized eggs were obtained by injecting pairs of frogs with human chorionic gonadotropin (Sigma) by standard laboratory technique (BROWN, 1970). X. laevis embryos and larvae were staged according to NIEUWKOOP and FABER (1956). Embryos were dejellied in 3% cystein, 0.1% papain, pH 8.0, and then cultured in aged dechlorinated tap water in glass bowls with an inner diameter of 19 cm. Each bowl contained one liter of water and 100 embryos. Embryos at the desired stage were placed in one liter of an aqueous solution of the chemical to be tested. Larval stages were grown in dechlorinated tap water in 100 liter aquaria at a density of 1 animal per liter.

Only clutches of eggs which proved to be at least 95% fertile

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Bulletin of Environmental Contamination & Toxicology,
Vol. 18, No. 4 © 1977 by Springer-Verlag New York Inc.

were used. Each experimental group was always paired with control eggs obtained from the same mated pair of frogs. Embryos were scored as normal or abnormal by examination under a dissecting microscope (see GREENHOUSE, 1976d, for details).

N-phenyl-α-naphthylamine is 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 was extracted with hexane. The amount of amine in solution was then determined spectrophotometrically.

ED50 was estimated from a log-dose percent plot of the data (GOLDSTEIN, ARONOW, and KALMAN, 1974). Data was also evaluated by the method of LITCHFIELD and WILCOXON (1949). Their procedure entails plotting data on logarithmic-probability paper and fitting a straight line through the points using a modified Chi square test. The ED50 is estimated from this line. Confidence limits are calculated from the slope of the line, an exponent derived from the tabulated data, and a nomograph supplied by the authors.

RESULTS

N-phenyl-α-naphthylamine

Previous work has shown that exposure of X.laevis embryos and larvae to N-phenyl-α-naphthylamine produced abnormal development and/or death. The data reported below confirm and extend these findings. Table 1 and figures 1 and 2 summarize data from a series of determinations of the effect of exposure to N-phenyl-αnaphthylamine on viability of X. laevis larvae.

TABLE 1

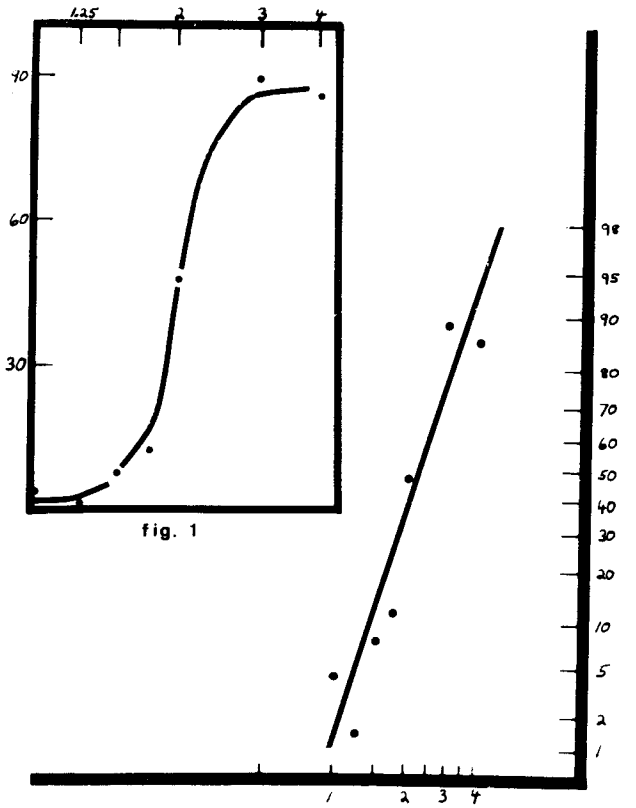
EFFECT OF N-PHENYL-α-NAPHTHYLAMINE ON VIABILITY OF XENOPUS LAEVIS LARVAE

Larvae were exposed to N-phenyl-α-naphthylamine . After 48 hours the number of dead/total larvae was recorded.

Exp. No.	Concentration in mg/liter						
	1.00	1.25	1.50	1.75	2.00	3.00	4.00
1	0/100	2/100	0/25	13/44	77/77	52/52	5/5
2	0/10	0/10	2/100	0/25	20/30	0/5	10/10
3	0/10	0/10	3/10	2/100	15/27	10/10	10/10
4	7/20	0/10	8/10	0/10	0/5	0/5	1/5
5	0/10	0/11	0/10	8/9	0/5	11/11	
6	0/10		0/10		6/10	10/10	
7					24/53		
Totals	7/160	2/141	13/165	23/188	142/297	83/93	26/30
%Dead	4.3	1.4	7.8	12.2	47.8	89.2	86.6

A log-dose percent lethality plot of the data (fig.1) in table 1 yields an estimated LD50 of 2.1 mg/liter. A log-dose probability

plot of the data (fig.2) yields an estimated LD50 of 2.3 mg/liter with 95% confidence limits between 1.96 and 2.76 mg/liter.



EFFECT OF N-PHENYL-α-NAPHTHYLAMINE ON VIABILITY OF XENOPUS LAEVIS LARVAE

Figure 1, Log-concentration percent mortality plot of the pooled data in table 1. Ordinate=percent killed. Abscissa=concentration.

Figure 2, Log-concentration-probability plot of the pooled data in table 1. Ordinate=percent killed. Abscissa=concentration.

Table 2 summarizes data on the teratogenic effect of N-phenyl-α-naphthylamine on Xenopus embryos. Embryos were continuously exposed to this compound from blastula until hatching at which time the number of malformed embryos/total number of embryos exposed was recorded.

TABLE 2

TERATOGENIC EFFECT OF N-PHENYL-α-NAPHTHYL-AMINE ON XENOPUS LAEVIS EMBRYOS

Exp. No.	Concentration mg/liter						
	3.0	4.0	4.5	5.3	6.0	6.8	7.5
1	15/100	34/100	100/100	148/292	80/85	17/17	73/73
2		18/105	17/100	116/219	87/91		10/10
3		4/79	41/82	0/50	115/115		
4					21/21		
Totals	15/100	56/284	158/282	264/561	303/312	17/17	83/83
%Malf.	15	20	56	47	97	100	100

A log-dose percent plot of the data in table 2 yields an estimated ED50 (teratogenesis) of 4.8 mg/liter (fig.3). A log-dose probability plot of the pooled data in table 2 yields an estimated ED50 of 4.57 mg/liter with 95% confidence limits between 3.93 and 5.30 mg/liter (fig.4).

Hydrazine

Table 3 and figures 5 and 6 summarize data on the teratogenicity of hydrazine. Embryos were continuously exposed to aqueous solutions of hydrazine from blastula until hatching at which time the number of malformed embryos/total number of embryos exposed was recorded.

TABLE 3

TERATOGENIC EFFECT OF HYDRAZINE ON XENOPUS EMBRYOS

Exp. No.	Concentration mg/liter			
	10	15	20	25
1	61/100	83/100	99/100	100/100
2	2/100			100/100
3				100/100
4				50/50
Totals	63/200	83/100	99/100	350/350
%Malf.	32	83	99	100

A log-dose percent plot of the pooled data in table 3 yields an estimated ED50 of 12.5 mg/liter (fig.5). An estimated ED50 of 11.48 mg/liter with 95% confidence limits between 10.95 and 12.03 mg/liter is obtained from the log-dose probability plot of the pooled data in table 3 (fig.6).

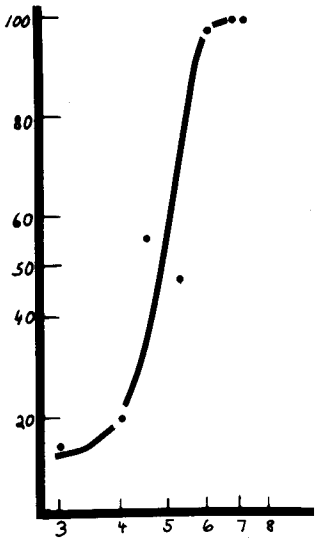


fig. 3

fig. 4

TERATOGENIC EFFECT OF N-PHENYL-α-NAPHTHYL-AMINE ON XENOPUS LAEVIS EMBRYOS

Figure 3, Log-concentration percent malformed plot of the pooled data in table 2. Ordinate=percent malformed. Abscissa=concentration.

Figure 4, Log-concentration probability plot of the pooled data in table 2. Ordinate=percent malformed. Abscissa=concentration.

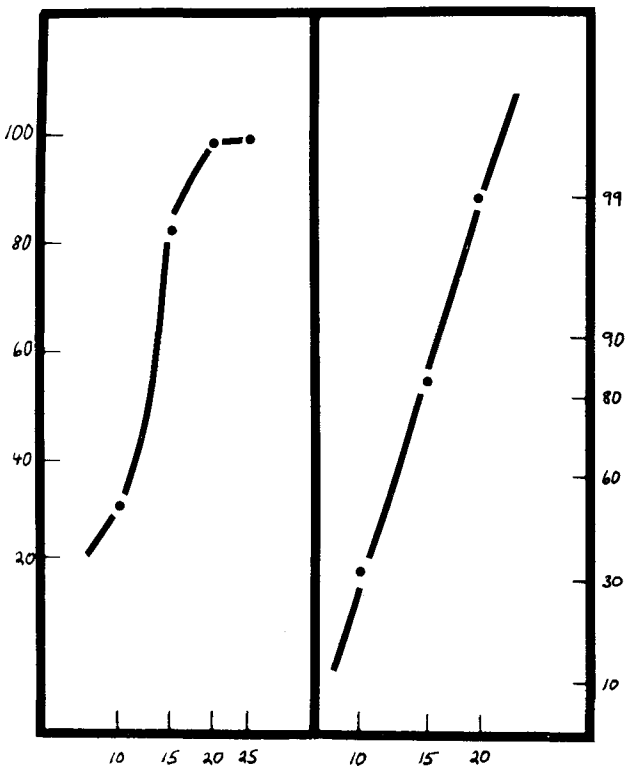


fig. 5 fig. 6

TERATOGENIC EFFECT OF HYDRAZINE ON XENOPUS LAEVIS EMBRYOS

Figure 5, Log-concentration percent malformed plot of the pooled data in table 3. Ordinate=percent malformed. Abscissa=concentration.

Figure 6, Log-concentration probability plot of the pooled data in table 3. Ordinate=percent malformed. Abscissa=concentration.

1,1-Dimethylhydrazine

Data on the teratogenicity of this compound is summarized in table 4 and figure 7 and 8. Embryos were continuously exposed to solutions of dimethylhydrazine from blastula until hatching at which time the number of malformed embryos /total number of embryos exposed was recorded.

TABLE 4

TERATOGENIC EFFECT OF 1,1-DIMETHYLHYDRAZINE ON XENOPUS LAEVIS EMBRYOS

Exp. No.	Concentration mg/liter		
	5	10	20
1	0/50	58/157	41/48
2	100/100	36/50	100/100
3	0/50	67/100	47/50
4	0/50		
Totals	100/250	161/307	188/198
%Malf.	40	52	95

The log-dose percent teratogenicity plot (fig.7) of the pooled data from table 4 yields an estimated ED50 of 9 mg/liter. The log-dose probability plot of the pooled data from table 4 yields an estimated ED50 of 7 mg/liter with 95% confidence limits between 3 and 17 mg/liter (fig.8).

DISCUSSION

The compounds tested were designated as potential environmental pollutants by the United States Air Force. Hydrazine and 1,1-dimethylhydrazine are components of rocket and jet fuels. The use of N-phenyl-α-naphthylamine was not specified.

My data on the toxicity of these compounds to frog embryos and larvae are similar to the results obtained by SCHERFIG and DIXON (1975) who used an Environmental Protection Agency approved algae system to assess the effects of these compounds on water quality. Both hydrazine and N-phenyl-α-naphthylamine were found to inhibit the growth of algae at approximately the same concentrations that affect the viability of tadpoles.

SUMMARY

The LD50 of N-phenyl-α-naphthylamine for X.laevis larvae was calculated to be 2.3 mg/liter with 95% confidence limits between 1.96 and 2.76 mg/liter.

The ED50 (teratogenesis) of N-phenyl-α-naphthylamine, hydrazine, and 1,1-dimethylhydrazine for X. laevis embryos was calculated to be:

Compound	ED50	95% Confidence
N-phenyl-α-naphthylamine	4.57 mg/liter	3.93- 5.30 mg/liter
Hydrazine	11.48 mg/liter	10.95-12.03 mg/liter
Dimethylhydrazine	7.00 mg/liter	3.00-17.00 mg/liter

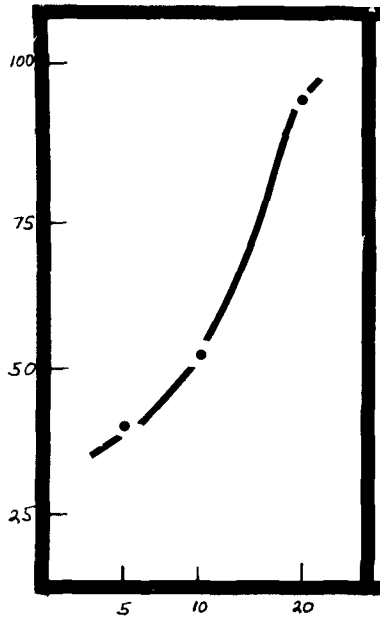


fig. 7

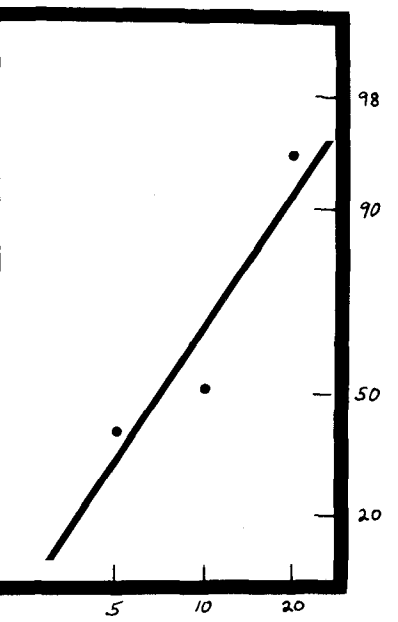


fig. 8

TERATOGENIC EFFECT OF 1,1-DIMETHYLHYDRAZINE ON XENOPUS LAEVIS EMBRYOS

Figure 7, Log-concentration percent malformed plot of the pooled data in table 4. Ordinate=percent malformed. Abscissa=concentration.

Figure 8, Log-concentration probability plot of the pooled data in table 4. Ordinate=percent malformed. Abscissa=concentration.

ACKNOWLEDGMENTS

This work was supported by contract AF F33615-C-76-5005 from the Aerospace Medical Division of the Air Force Systems Command to the University of California.

Technical assistance was provided by Ms. Penelope Kimbrell.

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