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National Toxicology Program

(Center)

National Institutes of Health
Bethesda, Maryland 20205

July 8, 1983

FYI-07J-0783-0251

INFO. CONTROL BRANCH
EPA
1983 JUL 13 AM 10:14

Mr. T. O'Bryan
Document Control Office
Management Support Division (TS-793)
Office of Toxic Substances
Environmental Protection Agency
401 M Street, S. W.
Washington, D. C. 20460

RECEIVED
67-10-83
PRE/STW
CSB

Dear Mr. O'Bryan:

This is in response to Mr. Frank Kover's letter requesting information on four chemicals, namely dimethyl methyl phosphonate, propionitrile, ziram, and methylcyclopentadienyl manganese tricarbonyl, for use by EPA in preparing Chemical Hazard Information Profiles (CHIPs) on the chemicals.

All of the chemicals except for methylcyclopentadienyl manganese tricarbonyl have been tested or are being tested by the National Toxicology Program (NTP) for one or more toxicological endpoints.

Dimethyl methyl phosphonate is in the chronic phase of a gavage carcinogenesis bioassay in rats and mice. It was negative in Salmonella strains TA98, TA100, TA1535, and TA1537 both with and without metabolic activation. It has been selected for testing in the mouse lymphoma assay and is on test for chromosomal aberrations and sister chromatid exchanges in cultured Chinese hamster ovary cells. The chemical is also on test in a sperm morphology assay. Enclosed are copies of the computer printouts containing the results of dimethyl methyl phosphonate in the four tester strains, as well as an outline of the Salmonella protocol and a sample computer printout containing explanations of all the entries in the printout. Also, enclosed is a copy of a letter to Dr. Bentley Gregg, Dynamac, from Dr. June Dunnick, chemical manager for the NTP carcinogenesis bioassay of dimethyl methyl phosphonate, listing the data on the chemical being forwarded to him. I understand from Hank Appleton, EPA, that Dr. Gregg is involved in preparing the CHIP on dimethyl methyl phosphonate for EPA.

Propionitrile has been selected for testing in the Salmonella assay as part of a class study of the genotoxic potential of nitriles in this test system. No other testing of the chemical has been scheduled.

BEST COPY AVAILABLE
ENTIRE DOCUMENT

Mr. T. O'Bryan

2

Ziram was positive when tested by the NTP in a feeding study in rats and mice, inducing increased incidences of C-cell carcinomas of the thyroid gland in male F344 rats. Although increased incidences of alveolar/bronchiolar adenomas and of combined alveolar/bronchiolar adenomas or carcinomas were observed in female B6C3F1 mice, the interpretation of these increases in lung tumors is complicated by an intercurrent Sendai virus infection. Hank Appleton has informed me that EPA already has a copy of the final technical report of the bioassay, so I am not enclosing another copy.

The chemical was positive in Salmonella strain TA100 with and without metabolic activation. It was also positive in strain TA1535 with hamster liver activation. It yielded positive and equivocal results in strain TA98 with hamster liver activation. Finally, ziram was negative in strain TA1537 both with and without metabolic activation. Enclosed are copies of the computer printouts of the results of ziram in the four tester strains.

If you have any questions regarding the NTP genotoxicity testing performed or presently underway on the three chemicals, kindly contact Dr. Errol Zeiger at FTS-629-4481. Please do not hesitate to contact me at 496-3511 if I can be of further assistance.

Sincerely yours,



Dorothy A. Canter, Ph.D.
Assistant to Director, NTP

Enclosures

cc: Dr. D. Rall
Dr. J. Moore
Dr. J. Dunnick
Dr. E. Zeiger

000003

June 10, 1983

Dr. Bentley Gregg
Dynamac Corporation
11140 Rockville Pike
Rockville, MD 20852

Dear Dr. Gregg:

The NTP subchronic study reports in mice and rats for Dimethyl Methyl Phosphonate are enclosed. A copy of the April 15, 1983, letter from Arthur D. Little, Inc., is also enclosed and specifies the reports they have prepared.

I hope this information is useful to you in preparing your report on DMMP for the Environmental Protection Agency. Please furnish the NTP with a copy of your completed report.

Sincerely,

June K. Dunnick, Ph.D.
Toxicologist
National Toxicology Program

Enclosures

JKD:sra

*cc Jeff
Schultz*

000004

ALQUOT #: 042521 DIMETHYL METHYLPHOSPHONATE 756-79-6
 EG&G MASOR RESEARCH INSTITUTE (LISTED BY EMIS SYSTEM) :

1

2

1 TAI00		NA (61010)				NA (61215)			
DOSE	U.	H2O		PREINC	SE	H2O		PREINC	SE
		A	B			A	B		
0.	100.	135	121	132	4.3	118	138	135	6.2
333.	1000.	130	142	141	3.8	119	149	136	8.7
3333.	10000.	132	142	140	3.1	125	103	123	7.0
		142	155	123	9.3	138	130	130	2.7
		154	139	132	6.5	116	132	138	6.6
		166	147	134	9.3	132	141	140	2.8

2 TAI00		RLI (61714)				RLI (61219)			
DOSE	U.	H2O		PREINC	SE	H2O		PREINC	SE
		A	B			A	B		
0.	100.	150	146	130	6.1	131	128	129	.9
333.	1000.	161	134	139	8.3	135	124	140	4.7
3333.	10000.	146	154	134	5.8	127	97	131	10.7
		155	161	138	6.9	153	134	132	6.7
		139	141	128	4.0	132	135	105	10.1
		142	133	131	3.4	133	113	130	4.6

3 TAI00		HLI (61018)				HLI (61223)			
DOSE	U.	H2O		PREINC	SE	H2O		PREINC	SE
		A	B			A	B		
0.	100.	123	128	123	1.7	125	140	131	4.4
333.	1000.	134	130	160	9.4	108.	125	120	5.0
3333.	10000.	119	136	157	11.0	106	129	122	6.8
		144	162	122	11.6	138	124	135	4.3
		145	135	104	11.1	140	145	120	7.6
		136	130	127	2.0	128	139	129	3.5

ALLOUT # 842521 DIMETHYL METHYLPHOSPHONATE 756-79-6
 EGAN MASON RESEARCH INSTITUTE (LISTED BY EMIS SYSTEM) :

1		2		3							
TA1535		TA1535		TA1535							
05/27/81		06/17/81		06/17/81							
(61011)		(61216)		(61224)							
NA	NA	NA	NA	NA	NA						
H2O	H2O	H2O	H2O	H2O	H2O						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
H2J	H2J	H2J	H2J	H2J	H2J						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
H2I	H2I	H2I	H2I	H2I	H2I						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
H2K	H2K	H2K	H2K	H2K	H2K						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
H2L	H2L	H2L	H2L	H2L	H2L						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
H2M	H2M	H2M	H2M	H2M	H2M						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
H2N	H2N	H2N	H2N	H2N	H2N						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
H2O	H2O	H2O	H2O	H2O	H2O						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
H2P	H2P	H2P	H2P	H2P	H2P						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
H2Q	H2Q	H2Q	H2Q	H2Q	H2Q						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
H2R	H2R	H2R	H2R	H2R	H2R						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
H2S	H2S	H2S	H2S	H2S	H2S						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
H2T	H2T	H2T	H2T	H2T	H2T						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
H2U	H2U	H2U	H2U	H2U	H2U						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
H2V	H2V	H2V	H2V	H2V	H2V						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
H2W	H2W	H2W	H2W	H2W	H2W						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
H2X	H2X	H2X	H2X	H2X	H2X						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
H2Y	H2Y	H2Y	H2Y	H2Y	H2Y						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
H2Z	H2Z	H2Z	H2Z	H2Z	H2Z						
A	A	A	A	A	A						
B	B	B	B	B	B						
PREINC	PREINC	PREINC	PREINC	PREINC	PREINC						
C	C	C	C	C	C						
MEAN	MEAN	MEAN	MEAN	MEAN	MEAN						
SE	SE	SE	SE	SE	SE						
DOSE	DOSE	DOSE	DOSE	DOSE	DOSE						
RLI	RLI	RLI	RLI	RLI	RLI						
31	24	24	26	2.3	29	27	27	2.4	21	26	2.4
22	33	21	25	3.8	21	30	30	2.6	25	25	2.6
19	25	30	27	5.0	19	26	20	2.5	18	21	2.5
30	21	27	26	2.6	29	30	20	3.2	20	26	3.2
29	22	33	28	3.2	21	25	29	2.3	29	25	2.3
23	21	36	27	4.7	20	25	21	1.5	21	22	1.5
10	10	20	13	3.3	14	13	8	1.9	8	12	1.9
9	14	16	13	2.1	10	11	10	.3	10	10	.3
13	12	9	11	1.2	13	11	14	.9	14	13	.9
15	11	14	13	1.2	10	16	13	1.7	13	13	1.7
13	9	12	11	1.2	8	10	9	.6	9	9	.6
19	10	14	14	2.6	9	14	7	2.1	7	10	2.1
15	11	7	11	2.3	14	13	14	1.5	9	12	1.5
7	9	4	8	.7	8	6	9	.9	9	8	.9
10	6	4	4	1.2	10	7	13	1.7	13	10	1.7
12	8	8	9	1.3	8	13	7	1.9	7	9	1.9
11	10	4	10	.6	12	6	7	1.9	7	8	1.9
13	11	11	12	.7	8	13	14	1.9	14	12	1.9

FOOTNOTES:

1. [4,1] SHARED WITH CHEMICALS 795473 AND 802373.
SHARED AS ABOVE.
2. [5,1] SHARED WITH CHEMICALS 795473 AND 802373.
SHARED AS ABOVE.
3. [11,1] SHARED WITH CHEMICALS 795473 AND 802373.
SHARED AS ABOVE.
4. [2,2] SHARED WITH CHEMICALS 023477, 758178, 795473, 802373, AND 843475.
SHARED AS ABOVE.
5. [11,2] SHARED WITH CHEMICALS 023477, 024472, 758178, 795473, AND 843475.
SHARED AS ABOVE.
6. [5,2] SHARED WITH CHEMICALS 023477, 758178, 795473, 802373, AND 843475.
SHARED AS ABOVE.

ALiquot #: 842521 DIMETHYL METHYLPHOSPHONATE 756-79-6
 EG&G MASON RESEARCH INSTITUTE (LISTED BY EMIS SYSTEM) :

2

1		TA1537 05/27/81		(61012)		TA1537 06/17/81		NA		(61217)	
DOSE	U.	PREINC		DOSE	U.	PREINC		A	B	PREINC	
		C	MEAN			SE	C			MEAN	SE
100.	100.	6	7	0.	0.	8	7	9	5	4	6
333.	333.	9	8	100.	100.	6	1.2	6	1	9	5
1000.	1000.	6	7	333.	333.	7	.3	8	4	4	5
3333.	3333.	5	4	1000.	1000.	4	.6	4	4	7	5
10000.	10000.	12	10	3333.	3333.	13	2.2	7	7	8	7
		8	6	10000.	10000.	3	1.5	8	8	8	8

2		TA1537 05/27/81		(61016)		TA1537 06/17/81		RLI		(61221)	
DOSE	U.	PREINC		DOSE	U.	PREINC		A	B	PREINC	
		C	MEAN			SE	C			MEAN	SE
100.	100.	9	8	0.	0.	8	.9	12	6	15	11
333.	333.	4	5	100.	100.	6	.7	8	13	6	9
1000.	1000.	3	7	333.	333.	6	2.3	7	8	6	7
3333.	3333.	7	7	1000.	1000.	6	.3	6	10	7	8
10000.	10000.	15	11	3333.	3333.	7	2.3	7	5	7	6
		7	8	10000.	10000.	9	.7	11	10	10	10

3		TA1537 05/27/81		(61020)		TA1537 06/17/81		HLI		(61225)	
DOSE	U.	PREINC		DOSE	U.	PREINC		A	B	PREINC	
		C	MEAN			SE	C			MEAN	SE
100.	100.	5	4	0.	0.	5	1.3	8	10	7	8
333.	333.	7	8	100.	100.	9	.6	10	6	12	9
1000.	1000.	7	8	333.	333.	8	.3	4	12	12	9
3333.	3333.	5	7	1000.	1000.	4	1.2	5	16	6	9
10000.	10000.	7	7	3333.	3333.	6	.6	12	12	4	9
		3	5	10000.	10000.	5	1.2	6	9	12	9

000008

ALiquot #: 842521 DIMETHYL METHYLPHOSPHONATE 756-79-6
 EGG MASON RESEARCH INSTITUTE (LISTED BY EMIS SYSTEM) :

2

1 TAYH NA H2O PREINC TA98 H2O PREINC (61214)

USE	A	H	C	MEAN	SE	USE	A	H	C	MEAN	SE
0	21	21	20	21	.3	0	17	15	26	19	3.4
100	20	18	23	20	1.5	100	16	14	13	14	.9
333	28	20	18	22	3.1	333	11	12	23	15	3.8
1000	26	12	24	21	4.4	1000	15	15	16	15	.3
3333	25	31	17	24	4.1	3333	10	24	12	17	3.5
10000	21	14	20	18	2.2	10000	11	28	15	18	5.1

2 TAYH RLI H2O PREINC TA98 H2O PREINC (61218)

USE	A	B	C	MEAN	SE	USE	A	B	C	MEAN	SE
0	31	26	37	31	3.2	0	25	31	28	28	1.7
100	30	44	39	38	4.1	100	20	31	19	23	3.8
333	35	40	30	35	2.9	333	18	16	30	21	4.4
1000	29	30	29	31	2.3	1000	23	31	38	31	4.3
3333	29	22	25	25	2.0	3333	24	31	27	27	2.0
10000	28	37	38	34	3.2	10000	20	18	23	20	1.5

3 TAYH HLI H2O PREINC TA98 H2O PREINC (61222)

USE	A	B	C	MEAN	SE	USE	A	B	C	MEAN	SE
0	32	26	35	31	2.6	0	30	26	35	30	2.6
100	32	35	23	30	3.0	100	20	28	28	27	.7
333	26	21	32	26	3.2	333	29	23	28	27	1.9
1000	34	30	28	32	3.1	1000	24	33	33	30	3.0
3333	29	37	33	33	2.3	3333	20	25	19	23	2.2
10000	20	26	29	25	2.0	10000	24	24	27	25	1.0

SALMONELLA PROTOCOL

- All chemicals tested and evaluated blind
- TA98, TA100, TA1535, TA1537
- Non-activation; Arbcior 1254 - induced Sprague-Dawley rat and Syrian hamster S-9
- Preincubation protocol (20 min. at 37°C)
- Solvent control;
Positive control;
5 doses, no greater than half-log intervals
- High dose determined by toxicity or solubility
- All plates in triplicate
- Incubated 37°C / 2 days
- Test repeated at least 1 week later

000010

ALiquot #: <CHEMICAL CODE NUMBER>
 <NAME OF CONTRACT LABORATORY>

<CHEMICAL NAME>

<CAS #>

TRIAL# - 1

NOT ACTIVATED
 (NO 5-9)

STRAIN 2

SOLVENT
 NA DMSO

PREINCUBATION
 PERCENT

EXPT. DATE
 1 TA1535
 10/08/80

(15335)

TA1535
 11/19/80

(15344) - COMPUTER
 ACCESS

DOSE	A	B	C	MEAN	SE
0.	17	30	26	24	3.8
33.	21	21	17	20	1.3
100.	17	23	27	22	2.9
333.	215	155	225	19	2.2
1000.	145	205	175	17	1.7
3333.	OT	OT	OT		

DOSE	A	B	C	MEAN	SE
0.	23	11	20	18	3.6
500.	9	22	18	16	3.8
750.	19	23	20	21	1.2
1000.	17	11	19	16	2.4
1500.	15P	22P	12P	16	3.0
2000.	19S	22S	10S	17	3.6

TOXIC RESPONSE
 ON PLATE (CLEAR
 BACKGROUND LAWN)

ARCLOR-INDUCED
 RAT LIVER S-9

RLI

(15336)

TA1535
 11/19/80

(15345)

DOSE	A	B	C	MEAN	SE
0.	9	5	16	10	3.2
100.	20	23	15	19	2.3
333.	17	C	22	20	1.5
1000.	32	22	30	28	3.1
3333.	16P	22P	23P	20	2.2
10000.	14P	18P	19P	16	1.2

CHEMICAL PRECIPITATED
 ON PLATE

EQUIVOCAL
 RESPONSE

POSITIVE
 RESPONSE

+

RLI

(15345)

DOSE	A	B	C	MEAN	SE
0.	9	12	12	11	1.0
500.	22	40	31	31	5.2
750.	31	44	37	37	3.8
1000.	28	35	15	26	5.9
1500.	13	28	25	22	4.6
2000.	41	20	24	28	6.4

ARCLOR-INDUCED
 HAMSTER LIVER S-9

HLI

(15337)

TA1535
 11/19/80

(15346)

DOSE	A	B	C	MEAN	SE
0.	11	11	10	11	.3
100.	10	7	12	10	1.5
333.	17	24	12	18	3.5
1000.	17	7	13	12	2.9
3333.	20P	17P	14P	17	1.7
10000.	10P	14P	16P	13	1.8

NEGATIVE
 RESPONSE

-

HLI

(15346)

DOSE	A	B	C	MEAN	SE
0.	11	9	18	12	3.2
500.	12	13	16	14	1.2
750.	11	24	22	19	4.0
1000.	15	13	7	12	2.4
1500.	17	11	15	14	1.8
2000.	16	12	21	16	2.6

000011

ALLOQUOT #: 356510 ZIRAM 137-30-4
 SKI INTERNATIONAL (LISTED BY EMIS SYSTEM) :

2

1 TAYH		NA		DMSO		PREINC		(41570)	
DOSE		A	B	C	MEAN	SE			
0.		33	20	17	23	4.9			
3.		13	21	17	17	2.3			
10.		31	24	17	24	4.0			
33.		44	25	25	31	6.3			
100.		32	29	37	33	2.3			
333.		35	35	215	9	6.0			

2 TAYH		RLI		DMSO		PREINC		(41574)	
DOSE		A	B	C	MEAN	SE			
0.		39	39	29	36	3.3			
3.		51	30	27	36	7.5			
10.		41	41	29	37	4.0			
33.		55	36	33	41	6.9			
100.		41	41	57	46	5.3			
333.		205	285	375	28	4.9			

3 TAYH		HLI		DMSO		PREINC		(41578)		TA98		HLI		DMSO		PREINC		(41716)	
DOSE		A	B	C	MEAN	SE				DOSE	A	B	C	MEAN	SE				
0.		31	24	28	28	2.0				0.	28	30	30	29	.7				
3.		32	32	21	28	3.7				3.	44	49	32	42	5.0				
10.		29	37	31	32	2.4				10.	46	54	36	46	5.3				
33.		31	29	33	31	1.2				33.	50	44	0C	47	3.0				
100.		61	55	53	56	2.4				100.	89	78	90	86	3.8				
333.		195	265	305	25	3.2				333.	100	545	535	69	15.5				

ALLOUT # : J50540 ZIRAN 137-30-4
 SKI INTERNATIONAL (LISTED BY EMIS SYSTEM) :

1

1 TA1537 NA DMSU PREINC (41569)

DOSE	A	B	C	MEAN	SE
0.	4	4	6	5	.7
3.	5	3	9	6	1.8
10.	9	7	6	7	.9
33.	7	8	2	6	1.9
100.	5	15	15	12	3.3
333.	95	145	05	H	4.1

2 TA1537 RLI DMSU PREINC (41573)

DOSE	A	B	C	MEAN	SE
0.	9	9	6	8	1.0
3.	14	9	15	13	1.9
10.	13	14	14	14	.3
33.	16	8	20	15	3.5
100.	15	28	17	20	4.0
333.	85	85	45	7	1.3

3 TA1537 HLI DMSU PREINC (41577)

DOSE	A	B	C	MEAN	SE
0.	8	12	6	9	1.8
3.	6	6	8	7	.7
10.	9	9	8	9	.3
33.	25	18	8	17	4.9
100.	13	16	21	17	2.3
333.	85	145	35	H	3.2

ALLOQUOT #: 350540 ZIRAM 137-30-4
 SKI INTERNATIONAL (LISTED BY EMIS SYSTEM) :

2

1

1 TA1535		VA		DMSD		PREINC		(41508)	
00/04/1981		DUSE		B		C		SE	
U.	3.	10.	33.	100.	333.	28	31	29	29
				21	20	24	27	22	27
				20	25	28	24	24	23
				21	40	30	32	32	5.8
				05	205	175	12	12	6.2

2 TA1535		RLI		DMSD		PREINC		(41572)	
00/04/1981		DUSE		B		C		SE	
U.	3.	10.	33.	100.	333.	12	14	13	13
				13	15	9	12	12	1.8
				12	15	9	12	12	1.7
				19	16	21	19	19	1.5
				29	20	21	23	23	2.8
				05	85	145	7	7	4.1

3 TA1535		HLI		DMSD		PREINC		(41576)		TA1535		PREINC		(41715)	
00/04/1981		DUSE		d		C		SE		06/17/1981		C		SE	
U.	3.	10.	33.	100.	333.	8	12	14	11	1.8	0.	17	15	16	.6
				12	4	5	7	5	7	2.5	3.	8.	12	8	2.0
				14	13	19	15	19	15	1.9	10.	5	9	9	2.6
				27	24	41	31	41	31	5.2	33.	27	21	24	1.7
				33	30	40	34	40	34	3.0	100.	28	53	41	7.3
				05	145	145	9	145	9	4.7	333.	255	435	32	5.6

ALLOUT #: 356540 ZIRAM 137-30-4
 DATA INTERNATIONAL (LISTED BY EMIS SYSTEM) :

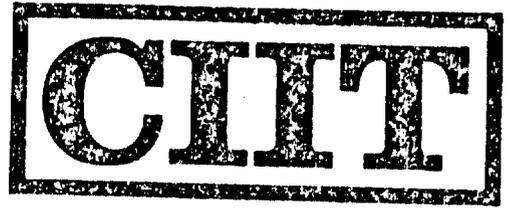
2

1 TAI00		NA (41571)			TAI00			NA (41713)		
06/04/1981		DMSO			DUSE			DMSO		
DUSE	A	B	C	MEAN	SE	06/17/1981	A	B	C	PREINC
0.	117	127	113	119	4.2	0.	152	172	174	166
3.	128	163	159	150	11.1	3.	197	174	160	177
10.	182	220	175	192	14.0	10.	263	262	284	270
33.	220	270	258	249	15.1	33.	375	369	379	374
100.	320	289	319	309	10.2	100.	543	502	470	505
333.	9RS	28S	9S	45	27.1	333.	469	295	286	350

2 TAI00		RLI (41575)			TAI00			RLI (41714)		
06/04/1981		DMSO			DUSE			DMSO		
DUSE	A	B	C	MEAN	SE	06/17/1981	A	B	C	PREINC
0.	136	110	117	121	7.8	0.	149	179	139	156
3.	178	160	177	172	5.8	3.	158	222	165	182
10.	173	201	218	197	13.1	10.	265	272	218	252
33.	288	294	252	288	3.5	33.	328	325	342	332
100.	323	375	323	340	17.3	100.	483	610	371	488
333.	167S	164S	140S	170	15.2	333.	280	445	283	336

3 TAI00		HLI (41579)			TAI00			HLI (41717)		
06/04/1981		DMSO			DUSE			DMSO		
DUSE	A	B	C	MEAN	SE	06/17/1981	A	B	C	PREINC
0.	105	105	125	112	6.7	0.	134	158	122	138
3.	179	125	115	140	19.9	3.	148.	137	142	142
10.	172	151	154	159	6.6	10.	0	175	185	120
33.	335	246	289	290	25.7	33.	172	330	328	277
100.	469	433	544	462	32.7	100.	259	580	587	475
333.	200S	348S	275S	274	42.7	333.	603	690	501	600

File with FYI-0251-



Chemical Industry Institute of Toxicology

President, Robert A. Neal, Ph.D.
Vice President, Director of Research, James E. Gibson, Ph.D.
Vice President, Administration and Secretary, Donald A. Hart, Ed.D.

P. O. Box 12137
Research Triangle Park,
North Carolina 27709
(919) 541-2070

James E. Gibson

June 21, 1983

Mr. Frank D. Kover
Chief, Chemical Hazard Identification
Branch (TS-778)
Office of Pesticides and Toxic Substances
U. S. Environmental Protection Agency
Washington, DC 20460

*6/14/83
CIIT
James E. Gibson
Vice President*

Dear Mr. Kover:

CIIT has not carried out any studies of the potential toxic effects of dimethyl methyl phosphonate or any other of the compounds noted in your recent letter. In addition, none of these compounds are on our priority chemicals list. Therefore, I do not contemplate that we will be carrying out any studies on these compounds in the foreseeable future.

If we can be of further assistance to you, please do not hesitate to contact us at any time.

Sincerely,

Robert A. Neal
Robert A. Neal
President

RAN:ewb



American Cyanamid Company
One Cyanamid Plaza
Wayne, NJ 07470

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FYI-OTS-0783-0251 Suppl.
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July 8, 1983

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Document Control Officer
Management Support Division (TS-793)
Office of Toxic Substances
401 M Street, S.W.
Washington, DC 20460

Attention: T. O'Bryan

Reference: Propionitrile (CAS 107-12-0)

Gentlemen:

Per your recent request to the Chemical Manufacturers Association for health and safety data on propionitrile to be used to prepare a Chemical Hazard Information Profile, we are enclosing a copy of a toxicity report prepared October 28, 1953 (Our Report 73-95).

We trust that our submission will be of assistance in your evaluation of propionitrile.

Sincerely,

Allan E. Sherr, Ph.D
Manager Chemical Petitions and
Regulatory Affairs
Toxicology and Product Safety Dept.

AES:sa
05/1374g

000009

INTER-OFFICE CORRESPONDENCE

note

53-95

New York July 8, 1954

OFFICE DATE

TO: New York

ATT'N. OF: Dr. N. B. Sommer

- COPY TO:
- Dr. G. B. Ayers
 - Mr. H. A. Bostrom
 - Mr. W. R. Bradley (2)
 - Dr. B. W. Carey
 - Dr. J. J. Carnes
 - Dr. R. P. Chapman
 - Mr. F. M. Cowen
 - Mr. C. P. Davis
 - Dr. H. C. Halsted
 - Mr. E. K. Hunt
 - Mr. H. R. Huston
 - Mr. K. H. Klipstein
 - Dr. A. F. Mangelsdorff
 - Dr. E. H. Northey
 - Dr. J. H. Paden (2)
 - Dr. A. L. Peiker
 - Dr. J. C. Pullman
 - Dr. N. Righthand
 - Mr. J. A. Schmidlein
 - Dr. C. B. Shaffer
 - Dr. R. C. Swain
 - Dr. J. T. Thurston
 - Mr. R. F. Uncles
 - Mr. J. B. Williamson
 - Dr. J. H. Wolfsie

SUBJECT: PROPIONITRILE - Toxicity Report

REFERENCE: Food Research Laboratories, Inc.
Report: October 28, 1953



SUMMARY AND CONCLUSIONS

Propionitrile is a moderately toxic compound by ingestion and inhalation. The acute oral LD_{50} has been found to be approximately 64 mg/kg. An atmosphere saturated with vapor killed all animals after an exposure of one hour. It penetrates the skin readily to produce systemic effects. By skin penetration the single dose LD_{50} has been found to be approximately 128 mg/kg. This places the compound in the category of a LAPI poison. The compound is not a strong primary irritant to the skin or eyes. The hazard from ingestion and skin contact appears to be equal to that of acrylonitrile, and in these respects it should be handled with all of the precautions accorded the latter compound. The hazard from inhalation is only slightly less than that of acrylonitrile because of the difference in vapor pressure.

Single or repeated contact with propionitrile by any route of exposure whatsoever should be avoided. Splashes of the material on skin or in eyes should be removed promptly by thorough washing.

Propionitrile

July 8, 1954

DETAILED REPORT

SAMPLE: The sample was received by Food Research Laboratories August 27, 1953, and assigned their Serial No. 67576. It was a colorless liquid identified by ACCO Code No. S-476-150.

SINGLE ORAL DOSES: The reference report shows propionitrile to be a moderately toxic substance in single doses by mouth to male albino rats, with an approximate LD₅₀ of 64 (44-93) mg/kg. Death from single oral doses was prompt; accelerated respiration and prostration were the most prominent symptoms observed. Survivors were held for a 7-day observation period, following which they were sacrificed and autopsied. No unusual gross pathology was found.

SKIN ABSORPTION AND IRRITATION: Propionitrile is a LAPI poison by skin contact. In the present investigation the undiluted material was applied to the closely-clipped abdomen of the albino rabbit under a cuff of plastic sheeting which encircled the trunk of the animal. The period of contact was 24 hours. Groups of 4 animals were tested at 64 mg/kg, 128 mg/kg and 256 mg/kg respectively, and the LD₅₀ was calculated to be 128 (86-191) mg/kg. At the lower dosage levels, moderate edema and erythema of the skin were observed at the end of the 24-hour period. However, at the highest dosage level, which was fatal to all animals, no gross skin lesions were seen externally, but at autopsy vascular dilation was observed in the skin and in other areas of the body. Spleens were dull in color and flaccid, livers were friable and the lungs were extremely hemorrhagic.

Note: Compare these findings with a similar study reported by Smyth et al in the A.M.A. Archives of Industrial Hygiene and Occupational Medicine 4: 119-22, 1951.

EYE IRRITATION: A 0.1 ml quantity of the compound was instilled into the conjunctival sac of the eyes of each of 6 rabbits, and 10 seconds thereafter the material was washed out with 20 ml of saline solution. Under these conditions, propionitrile was found to be only slightly irritating to the eye.

VAPOR INHALATION: A group of 6 albino rats was exposed to air substantially saturated with vapor of the compound for a period of 1 hour. The air-stream supplying the exposure chamber was passed at a rate of 4 l/min through a quantity of the

Propionitrile

July 8, 1954

compound contained in a gas-washing bottle, and in this manner 24 gm of the material was evaporated during the 1-hour exposure. The animals exhibited excessive salivation, accelerated respiration with gasping, prostration and narcosis. All of the animals died within 1 to 7 hours from the start of the exposure.

(Propionitrile has a vapor pressure of 40 mm Hg at 22°C. From this it may be calculated that air saturated with vapor of the compound at that temperature and 760 mm barometric pressure would contain approximately 120 mg/l [approximately 53,000 ppm] of propionitrile. It is possible to make a very rough estimate of the concentration attained in the 1-hour exposure from the rate of airflow and weight of material evaporated. This value is 100 mg/l.)

REPEATED INTAKE: Over a 28-day period, 3 groups of albino rats, 5 males and 5 females per group, were furnished drinking water containing propionitrile at the following concentrations: 2.5 mg/l, 12.5 mg/l and 62.5 mg/l, respectively. From records of water consumption, the mean daily intake of propionitrile is calculated as 0.32 mg/kg, 1.34 mg/kg and 3.72 mg/kg for the animals receiving the three concentrations, respectively. Over the 28-day period of the experiment, there were no significant differences between the groups receiving the two lower concentrations of the compound and their respective controls insofar as mortality, food intake and weight gain were concerned.

At the highest concentration of the compound in the drinking water (62.5 mg/l) there was one death. This occurred in the male group at 14 days, and hemorrhagic lungs were found in this animal at autopsy. The food consumption of both sexes at this level was somewhat less than the control intake, and the weight gain was quite significantly lower than that of the controls. There was definite impairment of efficiency of food utilization.

H. H. Golz, M.D.

HHG:ljs

000005

The Linear Free-Energy Relationship between Partition Coefficients and the Aqueous Solubility of Organic Liquids¹

CORWIN HANSCH,² JOHN E. QUINLAN, AND GARY L. LAWRENCE

Department of Chemistry, Pomona College, Claremont, California 91711

Received July 14, 1967

In this report it is shown that there is a linear relationship between the logarithms of the aqueous solubilities of organic liquids and their octanol-water partition coefficients. Since the logarithm of the partition coefficient is an additive-constitutive property of organic molecules, the water solubility of liquids whose partition coefficients have not been measured can be calculated. Or, one can simply construct a table of constants for the water solubility of organic liquids analogous to parachor values or molar refractivities. A problem of increasing importance in physical biochemistry and pharmacology is the selection of a suitable solvent pair (water and an apolar liquid) to serve as a model for the aqueous and lipid phases in biological systems. We have been using octanol-water partition coefficients for whole molecules or parts of molecules to estimate relative strengths of hydrophobic bonding in such systems. The present study would indicate that, with the exception of hydrocarbons, one could expect similar results from almost any monofunctional liquid, such as an alcohol, alkyl halide, ester, ketone, etc., representing the lipid phase.

Since the classic work of Meyer³ and Overton,⁴ scientists concerned with the correlation of chemical structure with biological activity have been seeking suitable solvents to approximate the aqueous and fatty phases of living tissue. We have been using 1-octanol and water to obtain partition coefficients which could serve as hydrophobic bonding constants. When combined with suitable electronic and steric constants, these would form the basis for a multiple-parameter approach to structure-activity relationships in biochemical systems.⁵

Following the lead of Hammett⁶ and Taft,⁶ we have formulated⁷ a substituent constant defined as in eq 1,

$$\pi_x = \log P_x - \log P_H \quad (1)$$

where P_x is the partition coefficient of a derivative and P_H that of the parent molecule. Thus π is proportional to the free energy of transfer of the function X from one phase to another. Our work, as well as that of others,⁸ has shown π to be an additive-constitutive property of organic compounds. We have shown that it can be used to account for the hydrophobic forces involved in the binding of organic compounds by proteins^{9c} and enzymes.^{5b}

Because of the analogy between the dissolving of an organic liquid in water and its partitioning between two solvents, it occurred to us that $\log P$ and π might be of use in correlating chemical structure with aqueous solubility.

The equilibrium between an organic liquid and its saturated aqueous solution may be thought of as the partitioning of the organic compound between itself

and water. We therefore sought a linear relationship between the free-energy changes for the two kinds of partitioning represented by S and P in eq 2, where S is

$$\log \frac{1}{S} = a \log P + b \quad (2)$$

the molal solubility of the organic liquid in water and P is its partition coefficient between 1-octanol and water. Fitting the data in Table I to eq 2 by the method of least-squares yields the sets of slopes and intercepts in Table II. The correlation coefficient is represented by r and s is the standard deviation of the regression of $\log 1/S$ on $\log P$. The numbers following the slopes and intercepts are the 90% confidence intervals on these quantities.

All of the correlations are quite good, especially when one considers that only a small fraction (22 out of 156) of the partition coefficients were actually measured and that the solubility data were taken from the work of many investigators whose results were obtained by different techniques on compounds of various degrees of purity over a temperature range of 15–25°. The slopes of sets 1–9 are remarkably similar; the 90% confidence intervals all overlap or come very close to the slope 1.21 of set 10.

Most interesting are sets 10 and 11. Set 10 correlates the solubility of 140 liquids (alkanes excluded) with about as much precision as one could expect, considering the nature of the data. The equation with these constants accounts for 91% ($r^2 = 0.91$) of the variance in the data, leaving only 9% to imperfections in the mathematical model and experimental error in measuring S and calculating $\log P$. The hydrocarbons behave somewhat differently as indicated by the intercept in set 9. The correlations embodied in set 1–11 show that the solubility of organic liquids in water is susceptible to evaluation by a Hammett-type substituent constant, linear, free-energy relationship.

Experimental Section

Only for those compounds in Table I marked by asterisks were experimentally determined partition coefficients used. Partition coefficients for the other compounds were calculated taking advantage of the additive nature of π and $\log P$. The π values for CH_3 and CH_2 were taken as 0.50; for a double bond 0.30 was subtracted from the value for the corresponding

(1) This work was supported by Research Grant GM-07492 from the National Institutes of Health.

(2) John Simon Guggenheim Fellow.

(3) H. Meyer, *Arch. Exptl. Pathol. Pharmacol.*, **43**, 109 (1899).

(4) E. Overton, *Vierteljahrschr. Naturforsch. Ges. Zuerich*, **44**, 88 (1899).

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E. W. Deutch and C. Hansch, *Nature*, **211**, 75 (1966); (e) C. Hansch and

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(7) T. Fujita, J. Iwasa, and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964).

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(b) C. Hansch and S. M. Anderson, *J. Org. Chem.*, **32**, 2583 (1967); (c) D. J.

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(1966); (d) P. Bracha and R. D. O'Brien, *J. Econ. Entomol.*, **59**, 1255 (1966).

TABLE I
 CORRELATION OF WATER SOLUBILITY WITH PARTITION COEFFICIENTS

Compound	Log P ^a	Log $\frac{1}{S}$		Compound	Log P ^a	Log $\frac{1}{S}$	
		obsd	calcd			obsd	calcd
1. Butanol	0.84	-0.026 ^c	0.169	68. Ethyl hexanoate	2.73	2.356 ^a	2.464
2. 2-Methyl-1-propanol	0.61	-0.098 ^d	-0.110	69. Ethyl heptanoate	3.23	2.737 ^a	3.071
3. 2-Butanol	0.61 [*]	-0.285 ^d	-0.110	70. Ethyl octanoate	3.73	3.387 ^a	3.678
4. Pentanol	1.34	0.592 ^c	0.776	71. Ethyl nonanoate	4.23	3.796 ^a	4.285
5. 3-Methyl-1-butanol	1.14	0.507 ^a	0.534	72. Ethyl decanoate	4.73	4.097 ^a	4.892
6. Methylbutanol	1.14	0.460 ^a	0.534	73. Diethyl ether	1.03	0.063 ^a	0.400
7. 2-Pentanol	1.14	0.276 ^a	0.534	74. Methyl butyl ether	1.53	0.992 ^a	1.007
8. 3-Pentanol	1.14	0.211 ^a	0.534	75. Methyl isobutyl ether	1.33	0.899 ^a	0.764
9. 3-Methyl-2-butanol	0.91	0.176 ^a	0.254	76. Methyl sec-butyl ether	1.33	0.734 ^a	0.764
10. 2-Methyl-2-butanol	0.89 [*]	-0.147 ^a	0.230	77. Methyl t-butyl ether	1.06	0.210 ^a	0.437
11. 2,2-Dimethylpropanol	1.36 [*]	0.386 ^a	0.801	78. Ethyl propyl ether	1.53	0.665 ^a	1.007
12. Hexanol	1.84	1.212 ^c	1.383	79. Ethyl isopropyl ether	1.33	0.554 ^a	0.764
13. 2-Hexanol	1.61	0.867 ^f	1.104	80. Dipropyl ether	2.03	1.317 ^a	1.614
14. 3-Hexanol	1.61	0.795 ^f	1.104	81. Propyl isopropyl ether	1.83	1.335 ^a	1.371
15. 3-Methyl-3-pentanol	1.39	0.361 ^f	0.837	82. Methyl propyl ether	1.03	0.372 ^a	0.400
16. 2-Methyl-2-pentanol	1.39	0.485 ^f	0.837	83. Methyl isopropyl ether	0.83	0.028 ^a	0.157
17. 2-Methyl-3-pentanol	1.41	0.697 ^f	0.861	84. Cyclopropyl ethyl ether	1.24	0.638 ^a	0.655
18. 3-Methyl-2-pentanol	1.41	0.713 ^f	0.861	85. Chloroethane	1.39	1.051 ^f	0.837
19. 4-Methyl-2-pentanol	1.41	0.787 ^f	0.861	86. Chloropropane	1.89	1.527 ^f	1.444
20. 2,3-Dimethyl-2-butanol	1.17	0.370 ^f	0.570	87. 2-Chloropropane	1.69	1.358 ^f	1.201
21. 3,3-Dimethyl-1-butanol	1.86	1.125 ^f	1.408	88. Chlorobutane	2.39 [*]	2.143 ^f	2.051
22. 3,3-Dimethyl-2-butanol	1.19	0.613 ^f	0.594	89. Isobutyl chloride	2.19	2.000 ^f	1.808
23. Heptanol	2.34	1.809 ^c	1.990	90. 1,3-Dichloropropane	2.28	1.614 ^a	1.918
24. 2-Methyl-2-hexanol	1.87	1.074 ^a	1.420	91. Chloroform	1.97 [*]	0.920 ^f	1.541
25. 3-Methyl-3-hexanol	1.87	0.984 ^a	1.420	92. Bromoethane	1.60	1.055 ^f	1.092
26. 3-Ethyl-3-pentanol	1.87	0.832 ^a	1.420	93. Bromopropane	2.10 [*]	1.733 ^f	1.699
27. 2,3-Dimethyl-2-pentanol	1.67	0.871 ^a	1.177	94. 2-Bromopropane	1.90	1.631 ^f	1.456
28. 2,3-Dimethyl-3-pentanol	1.67	0.843 ^a	1.177	95. Bromobutane	2.60	2.366 ^f	2.306
29. 2,4-Dimethyl-2-pentanol	1.67	0.932 ^a	1.177	96. Isobutyl bromide	2.40	2.432 ^f	2.063
30. 2,4-Dimethyl-3-pentanol	1.71	1.217 ^a	1.226	97. Isoamyl bromide	2.90	2.886 ^f	2.670
31. 2,2-Dimethyl-3-pentanol	1.69	1.148 ^a	1.201	98. 1,3-Dibromopropane	2.70	2.081 ^f	2.428
32. Octanol	2.84	2.346 ^c	2.597	99. Iodomethane	1.50	1.000 ^f	0.971
33. 2,2,3-Trimethyl-3-pentanol	1.99	1.273 ^a	1.566	100. Iodoethane	2.00 [*]	1.600 ^f	1.578
34. Cyclohexanol	1.23 [*]	0.417 ^a	0.643	101. Iodopropane	2.50	2.290 ^f	2.185
35. 4-Penten-1-ol	1.04	0.154 ^f	0.412	102. Iodobutane	3.00	2.960 ^f	2.792
36. 3-Penten-2-ol	0.81	-0.055 ^f	0.133	103. Diiodomethane	2.50	2.340 ^f	2.185
37. 1-Penten-3-ol	0.81	-0.015 ^f	0.133	104. (CICH ₂ CH ₂) ₂ S	2.73	2.370 ^f	2.464
38. 1-Hexen-3-ol	1.31	0.588 ^f	0.740	105. 1-Pentyne	1.98 [*]	1.640 ^f	1.553
39. 2-Hexen-4-ol	1.31	0.403 ^f	0.740	106. 1-Hexyne	2.48	2.360 ^a	2.160
40. 2-Methyl-4-penten-3-ol	1.11	0.502 ^f	0.497	107. 1-Heptyne	2.98	3.010 ^a	2.767
41. Benzyl alcohol	1.10 [*]	0.454 ^f	0.485	108. 1-Octyne	3.48	3.660 ^a	3.374
42. 2-Butanone	0.29 [*]	-0.678 ^a	-0.498	109. 1-Nonyne	3.98	4.240 ^a	3.981
43. 2-Pentanone	0.79	0.169 ^a	0.109	110. 1,8-Nonadiyne	3.46	2.980 ^a	3.350
44. 3-Pentanone	0.79	0.232 ^a	0.109	111. 1,6-Heptadiyne	2.46	1.750 ^a	2.136
45. 3-Methyl-2-butanone	0.59	0.124 ^a	-0.134	112. 1-Pentene	2.20	2.670 ^a	1.820
46. 2-Hexanone	1.29	0.779 ^a	0.716	113. 2-Pentene	2.20	2.540 ^a	1.820
47. 3-Hexanone	1.29	0.827 ^a	0.716	114. 1-Hexene	2.70	3.230 ^a	2.428
48. 3-Methyl-2-pentanone	1.09	0.671 ^a	0.473	115. 2-Heptene	3.20	3.820 ^a	3.035
49. 4-Methyl-2-pentanone	1.09	0.711 ^a	0.473	116. 1-Octene	3.70	4.620 ^a	3.642
50. 4-Methyl-3-pentanone	1.09	0.812 ^a	0.473	117. 4-Methyl-1-pentene	2.50	3.240 ^a	2.185
51. 2-Heptanone	1.79	1.422 ^a	1.323	118. 1,6-Heptadiene	2.90	3.340 ^a	2.670
52. 4-Heptanone	1.79	1.444 ^d	1.323	119. 1,5-Hexadiene	2.40	2.690 ^a	2.063
53. 2,4-Dimethyl-3-pentanone	1.39	1.299 ^a	0.837	120. 1,4-Pentadiene	1.90	2.080 ^a	1.456
54. 5-Nonanone	2.79	2.575 ^d	2.537	121. Cyclopentene	1.75	2.100 ^a	1.274
55. Ethyl formate	0.23	-0.076 ^a	-0.571	122. Cyclohexene	2.16	2.580 ^a	1.772
56. Propyl formate	0.73	0.491 ^f	0.036	123. Cycloheptene	2.57	3.160 ^a	2.270
57. Methyl acetate	0.23	-0.517 ^f	-0.571	124. Benzene	2.13 [*]	1.637 ^f	1.736
58. Ethyl acetate	0.73 [*]	0.040 ^a	0.036	125. Toluene	2.69 [*]	2.292 ^f	2.415
59. Propyl acetate	1.23	0.733 ^f	0.643	126. Ethylbenzene	3.15 [*]	2.880 ^f	2.974
60. Isopropyl acetate	1.03	0.519 ^f	0.400	127. Propylbenzene	3.68 [*]	3.302 ^f	3.617
61. Butyl acetate	1.73	0.693 ^f	1.250	128. Fluorobenzene	2.27 [*]	1.796 ^f	1.905
62. Isobutyl acetate	1.53	1.237 ^f	1.007	129. Chlorobenzene	2.84 [*]	2.363 ^f	2.597
63. Methyl propionate	0.73	0.094 ^f	0.036	130. Bromobenzene	2.99 [*]	2.547 ^f	2.780
64. Methyl butyrate	1.23	0.779 ^f	0.643	131. Nitrobenzene	1.85 [*]	1.777 ^f	1.396
65. Ethyl butyrate	1.73	1.275 ^f	1.250	132. 1,2,4-Trimethylbenzene	3.65	3.320 ^a	3.581
66. Propyl butyrate	2.23	1.907 ^a	1.857	133. o-Xylene	3.15	2.780 ^a	2.974
67. Ethyl valerate	2.23	1.767 ^a	1.857	134. Isopropylbenzene	3.43	3.380 ^a	3.314

TABLE I (Continued)

Compound	Log P^*	Log $\frac{1}{S}$		Compound	Log P^*	Log $\frac{1}{S}$	
		obsd	calcd			obsd	calcd
135. <i>m</i> -Nitrotoluene	2.42	2.439 ^c	2.088	146. Heptane	3.50	4.530 ^e	4.577
136. <i>o</i> -Dichlorobenzene	3.55	3.006 ^c	3.459	147. 2,4-Dimethylpentane	3.10	4.390 ^e	4.082
137. <i>m</i> -Dichlorobenzene	3.55	3.077 ^c	3.459	148. 2,2-Dimethylpentane	3.10	3.670 ^e	4.082
138. Ethyl benzoate	2.62	2.280 ^c	2.330	149. Octane	4.00	5.240 ^e	5.195
139. Aniline	0.90*	0.410 ^c	0.242	150. Cyclopentane	2.05	2.650 ^e	2.783
140. Propionitrile	0.16*	-0.280 ^c	-0.656	151. Cyclohexane	2.46	3.180 ^e	3.290
141. Pentane	2.50*	3.270 ^c	3.340	152. Methylcyclopentane	2.35	3.300 ^e	3.154
142. Isopentane	2.30	3.180 ^c	3.092	153. Cycloheptane	2.87	3.510 ^e	3.797
143. 2-Methylpentane	2.80	3.790 ^c	3.711	154. Methylcyclohexane	2.76	3.850 ^e	3.661
144. 3-Methylpentane	2.80	3.830 ^c	3.711	155. Cyclooctane	3.28	4.150 ^e	4.304
145. Hexane	3.00	3.960 ^c	3.958	156. 1,2-Dimethylcyclohexane	3.06	4.270 ^e	4.032

* S is the molal concentration. ^b Calculated values for compounds 1-140 were made using constants in set 10. Values for compounds 141-156 were found using constants in set 9. ^c J. A. V. Butler, D. W. Thompson, and W. H. Maclennan, *J. Chem. Soc.*, 674 (1933). ^d S. R. Palit, *J. Phys. Chem.*, 51, 837 (1947). ^e P. M. Ginnings and R. Baum, *J. Am. Chem. Soc.*, 59, 1111 (1937). ^f P. M. Ginnings and R. Webb, *ibid.*, 60, 1388 (1938). ^g P. M. Ginnings and M. Hauser, *ibid.*, 60, 2581 (1938). ^h P. M. Ginnings and D. Coltrane, *ibid.*, 61, 525 (1939). ⁱ A. Seidell, "Solubilities of Organic Compounds," Vol. 2, 3rd ed, D. Van Nostrand Co., New York, N. Y., 1941. ^j P. M. Ginnings, E. Herring, and D. Coltrane, *J. Am. Chem. Soc.*, 61, 807 (1939).

alkane⁷ and for a branch in the chain (e.g., isopropyl vs. *n*-propyl) 0.20 was subtracted from the value for the straight-chain compound. The value for cyclohexane was estimated in two ways. Subtracting log P for phenoxyacetic acid from 4-cyclohexylphenoxyacetic acid⁶ gives log P for cyclohexane as 2.51 (hydrogen is taken as zero). The π value for OH is -1.16 (log P *n*-propyl alcohol -1.50); subtracting this from log P for cyclohexanol yields 2.39. We have chosen the value of 2.46 for cyclohexane and hence the value of 0.41 for each cyclic CH₂ group. Other cycloparaffins were calculated by adding or subtracting 0.41 for each CH₂ to cyclohexane. Log P for (ClCH₂CH₂)₂S, mustard gas, was calculated by adding 2(0.39), π for 2 Cl, to log P of 1.95 for diethyl sulfide. π for Cl is found by subtracting 2.00 (log P CH₃CH₂CH₂CH₂-) from log P of 2.39 for 1-chlorobutane. The values for the alcohols were based on 1-propanol (log P 0.34) and *t*-butyl alcohol (log P 0.37) in addition to the values marked in Table I. The values for the ethers were based on log P 2.03 for ethyl butyl ether. The example of compound 40, Table I, serves to illustrate the method of calculation. To the value of 0.61 for 2-butanol was added 1.00 for the two additional carbon atoms. From 1.61 was subtracted 0.20 for a branch in the chain and 0.30 for the double bond to obtain the figure of 1.11. Cyclopropyl ether was calculated as follows. Subtraction of 2.00 from 2.03 for butyl ethyl ether yields the value of 0.03 for the -OEt fragment. Subtracting 0.29 for 2-butanone from the value⁷ of 1.50 for CH₃COCH₂CH₂-C₂H₅ yields a value of 1.21 for the cyclopropyl group. Thus 0.03 + 1.21 = 1.24 for compound 84, Table I.

The partition coefficients were determined as previously described.^{8a} Where possible, analysis of the phases was done using a Cary Model 14 spectrophotometer. For those molecules not adsorbing strongly in the ultraviolet region, vapor phase chromatography was used for analysis. Complete details of this latter method will be published elsewhere.

The values for log $1/S$ in Table I were selected, where possible, for solubility at 20-25°. In some instances several values were given for a particular compound at different temperatures and in these cases we interpolated to 25°.

Discussion

In considering the correlations obtained with sets 1-11, one must face the result that, except for the alkanes and possibly the alkenes, each of the liquids appears to partition between itself and water in much the same way as it partitions between octanol and water. That equations with constants of sets 10 and 11 should

hold can be rationalized from the thermodynamic point of view as follows. Consider the *i*th solute of a group whose solubilities and partition coefficients are being compared. For pure solute in equilibrium with a saturated aqueous solution we can equate the chemical potentials of the solute in the two phases. In eq 3,

$$\mu_i(l) = \mu_i^\circ(\text{H}_2\text{O}) + RT \ln S \quad (3)$$

$\mu_i(l)$ and $\mu_i^\circ(\text{H}_2\text{O})$ are the chemical potentials of the pure liquid solute and of the solute in a 1 *M* ideal aqueous solution, the hypothetical standard state for the solute. S is the molar concentration of solute in the saturated aqueous solution. This expression ignores any nonideality of the solution and the thermodynamic activity of the solute has been replaced by its molar concentration.

Similarly for the partitioning of the same solute between water and octanol, we have at equilibrium

$$\mu_i^\circ(\text{H}_2\text{O}) + RT \ln C_i(\text{H}_2\text{O}) = \mu_i^\circ(\text{oct}) + RT \ln C_i(\text{oct}) \quad (4)$$

where μ_i° has the same significance as in eq 3, in one case referring to the aqueous solution and in the other to the octanol solution. C_i refers to the molar concentration of the solute in each of the phases. Substituting P , the partition coefficient for the ratio $C_i(\text{oct})/C_i(\text{H}_2\text{O})$, eq 3 and 4 yield

$$\mu_i^\circ(\text{H}_2\text{O}) = \mu_i(l) - RT \ln S = \mu_i^\circ(\text{oct}) + RT \ln P \quad (5)$$

from which we obtain

$$\log \frac{1}{S} = \log P + \frac{\mu_i^\circ(\text{oct}) - \mu_i(l)}{2.303RT} \quad (6)$$

which is of the same form as eq 2. The last term in eq 6 is related to the free-energy change in dissolving 1 mole of pure solute in octanol to give a 1 *M* ideal solution. For the ideal solution the only contribution to this term is the entropy of mixing. The value of the intercept calculated on this basis is -1.28.

The octanol-water partition coefficients were all determined at low concentrations, 10⁻² to 10⁻³ *M* in

TABLE II

Type of compd	No. of compd ^a	Slope ^b	Intercept ^c	r	r^2
1. Alcohols	1-41	1.113 ± 0.08	-0.926 ± 0.12	0.967	0.136
2. Ketones	42-54	1.229 ± 0.13	-0.720 ± 0.19	0.980	0.164
3. Esters	55-72	1.013 ± 0.06	-0.520 ± 0.15	0.990	0.201
4. Ethers	73-84	1.182 ± 0.25	-0.935 ± 0.35	0.938	0.160
5. Alkyl halides	85-104	1.221 ± 0.20	-0.832 ± 0.45	0.928	0.235
6. Alkynes	105-111	1.294 ± 0.37	-1.043 ± 1.13	0.953	0.319
7. Alkenes	112-123	1.294 ± 0.13	-0.248 ± 0.33	0.985	0.131
8. Aromatics	124-139	0.996 ± 0.11	-0.339 ± 0.31	0.975	0.179
9. Alkanes	141-156	1.237 ± 0.18	0.248 ± 0.54	0.953	0.199
10. All compounds less alkanes	1-140	1.214 ± 0.05	-0.850 ± 0.11	0.955	0.344
11. All compounds	1-156	1.339 ± 0.07	-0.978 ± 0.15	0.935	0.472

^a Number of compound in Table II. ^b a in eq 3. ^c b in eq 2.

octanol and 10^{-3} to 10^{-4} M in water. The assumption of ideality in eq 4 is therefore reasonable. However, almost half of the compounds in Table I have solubilities exceeding 0.1 M and ten have solubilities greater than 1.0 M . The effect of considering non-ideality would be to add a term, $RT \ln \gamma_i$, to eq 3 and 6 where γ_i is the activity coefficient of the i th solute in its saturated aqueous solution. Inasmuch as most of these aqueous solutions would be expected to show positive deviations from Raoult's law, most values of γ_i would be greater than one. The correction term $RT \ln \gamma$ would therefore account for at least part of the difference between the contribution of the intercept of -1.28 due to entropy of mixing and the observed value of -0.85 in set 10.

We are concerned here with the common solubility-limiting characteristics of a large group of compounds. The dissolution of such compounds in water is a complex process and a variety of forces such as hydrogen bonding, dipole interactions, and dispersion forces have been recognized as factors which must be considered.⁹ The concept of the hydrophobic "bond" which has been developing from the studies of Frank and Evans¹⁰ seems to us to be useful in understanding the results.

The hydrophobic "bond" is complex, involving polar and apolar interactions. While the concept has been particularly fruitful in rationalizing biochemical phenomena,^{6,11-13} Scheraga¹⁴ and his coworkers have applied it to association of organic molecules in aqueous solution.

It has been shown that the transfer of a hydrocarbon from a nonpolar environment to an aqueous one is exothermic for aliphatic hydrocarbons and approximately athermal for aromatics. The low solubility of these and other organic compounds in water is associated with a large negative entropy of solution¹⁵ which is due to the formation of a loosely held but highly structured envelope of water molecules around the apolar portions of the organic molecules as they enter the solution. It is predominantly the molecular size and shape which determines how many water molecules enter into the structured sheath around the

apolar portions of the organic solute molecule and therefore determines the magnitude of the negative entropy of solution.

The linear free-energy relationships embodied in sets 1-11 as well as the constitutive and additive nature of π indicate that the major factor determining the partitioning of organic molecules between aqueous and organic phases is the extent to which they form hydrophobic bonds. *The effects of hydrophobic bonding so outweigh the various interactions of solute molecules with the organic member of the solvent system that the excellent correlations of sets 10 and 11 result.* Thus using these equations and π values derived from measurements of octanol-water partition coefficients for a limited number of organic compounds, it is possible to predict aqueous solubility of large numbers of organic liquids with quite satisfactory precision.

One of the important problems of structure-activity studies in biochemical systems is the selection of a suitable apolar liquid to model the lipid phases in biological systems. A suitable solvent pair such as water and 1-octanol could then be used as a reference system in the study of apolar interactions. Many different studies have been made attempting to correlate various kinds of biological responses with the way in which enzyme substrates or drugs distribute themselves between two phases. While many different solvents or combinations of solvents have been used, no extensive comparative studies of the relative value of different solvents have been made. The results in sets 1-11 would indicate that most monofunctional aliphatic liquids (except the alkanes) might give similar results.

In conclusion, one can say that the correlation obtained with the constants of set 10 justifies the assumptions made in deriving eq 6. As has been repeatedly pointed out,^{11,13} one of the justifications for studies of apolar interactions of small molecules with themselves is that it enables us to understand better their interactions with proteins and the internal bonding of proteins. In addition to showing that the water solubility of organic liquids is an additive-constitutive property, the present results help to clarify our understanding of the Meyer-Overton model using organic solvents to approximate biolipophilic phases.

Acknowledgment.—We are indebted to Susan M. Anderson, Smith Kline and French research associate, for the determination of a number of the partition coefficients used in this work.

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Dear Sir:

This responds to your request for information on a list of four chemical substances selected for preparation of Chemical Hazard Information Profiles (CHIP). We refer to a letter to Dr. Geraldine Cox of CMA, dated May 1983 from Frank D. Kover.

On 31 May 1983, Air Products and Chemicals, Inc. submitted to EPA a Health and Safety Studies Report entitled "Toxicity Screening Using MicrotoxTM" by Indurato, I.M., K. B. Snyder and P. J. Usinowicz. Although this report was submitted under provisions of 40 CFR 176, because it contained test data on toluenediamines, there is also test data on propionitrile included. A copy of the report is attached to this letter.

We appreciate the opportunity to provide input for your compilation of hazard data in the CHIPS' program.

Very truly yours,

C. E. Blades
C. E. Blades, Manager
Toxicology Department

CER:csb

Attachment

TDA

TOXICITY SCREENING USING MICROTOX™

Authors:

A. M. Indorato K. B. Snyder P. J. Usinowicz *

To Be Presented At:

FIRST INTERNATIONAL SYMPOSIUM ON TOXICITY TESTING USING BACTERIA

17 - 19 May 1983

To Be Held At:

THE NATIONAL WATER RESEARCH INSTITUTE

Burlington Ontario Canada

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P. O. Box 538 Allentown PA 18105

INTRODUCTION

Toxicity measurements are important in evaluating potential adverse environmental response of organisms to chemical exposure. For chemical wastewaters, discharge concentrations may be related to response of aquatic organisms, e.g. fish, daphnia, etc., reported as acute lethal concentrations resulting in fifty percent population reduction (LC_{50}). Testing is generally complicated and time-consuming. Simpler toxicity measurement techniques are useful in screening chemical components in proposed or existing wastewater discharges, and in decision making processes for wastewater handling. The Beckman MicrotoxTM EC_{50} (effective concentration resulting in ~~inactivation of~~ fifty percent of the bacteria, Photobacterium phosphoreum has been proposed as an alternative toxicity measurement. The usefulness, reliability, and relationships of EC_{50} to LC_{50} testing are important factors in application of EC_{50} toxicity data. Work performed in Air Products and Chemicals (APCI) laboratories using the EC_{50} measurements is reported herein.

PURPOSE OF STUDY

Three principal areas of interest were investigated:

- o Testing a variety of chemicals to determine EC_{50} and the effects of exposure time on the EC_{50} measurement.
- o Testing the MicrotoxTM as a screening device for EC_{50} reliability as a relative toxicity indicator.
- o Comparing and correlating EC_{50} with reported aquatic acute toxicity data (LC_{50}).

MICROTOX: THE INSTRUMENT

The MicrotoxTM, designed to operate with the Beckman reagent, is a precision photometric instrument. Using a marine luminescent bacterium (Photobacterium phosphoreum) the MicrotoxTM is sensitive to a wide variety of chemical compounds both organic and inorganic. To qualify the data reproducing capabilities of its instrument, Beckman ensures the genetic stability of its reagent. In addition, the routine use of a standard of known toxicity, in this case 2,6-dinitrophenol, provides a check on viability/consistency of the results.

The MicrotoxTM is simple in design and is easily calibrated and operated. The instrument includes: a fifteen compartment temperature controlled incubation area; a reaction chamber; and a digital display of percent light loss. There are also voltage and temperature checks, a chart recorder which graphically depicts percent light, a digital display, and system zero and temperature adjustments.

The theory of operation and instrumental procedures are discussed fully in Beckman technical literature (1).

DATA REDUCTION

A certain amount of light loss occurs naturally in the bacteria reagent; this is not due to any toxic effect. All data is calculated to compensate for this independent loss of light which occurs during the testing time.

The equation for this normalized light loss is (1):

$$\text{Normalized \% Light Loss} = \frac{\text{BR} (I_o) - (I_t) \times 100\%}{\text{BR} I_o}$$

where: BR = blank ratio

- this is derived by dividing the final time blank by the initial blank

$$\text{BR} = \frac{B_t}{B_o}$$

I_o = zero time light reading of any cuvette

I_t = final time light reading of any cuvette

Selected organic and inorganic compounds were chosen for toxicity testing by MicrotoxTM depending on availability of their LC_{50} data. Original reagents of 98% purity or less were further purified by simple distillation prior to MicrotoxTM testing. Sample concentrations were checked by total organic carbon analysis where applicable.

RESULTS AND DISCUSSION

Standard Toxicity Check

To insure the integrity of the MicrotoxTM, a standard of known toxicity (EC_{50}) is tested prior to any analyses of suspected toxins. The use of 2,6-dinitrophenol as a standard has been adopted.

Figure 1 illustrates the repeatability of results when the standard was tested. Also shown are two instances where grossly different results were seen. Since the instrument contains internal checking mechanisms, a change in results may indicate a problem with the reagent bacteria or a problem with the standard. In both cases shown, a new standard was prepared and replicate testing showed poor results, indicating that a problem existed in the reagent. The lot of bacteria was nearly outdated, and use of a fresh lot gave expected toxicity response for the standard.

Repeatability of EC₅₀ Testing

All EC₅₀ tests were done in duplicate to assure validity of results. Figure 2 shows duplicate test results for cyanide. Excellent agreement was obtained for cyanide at all exposure times tested. Figure 3 shows duplicate test results for 2,4-toluenediamine, and represents "worst-case" results obtained for the chemicals tested. Even this "worst-case" shows very good repeatability.

EC₅₀⁵ versus EC₅₀³⁰

EC₅₀ measurements were compared for each of the chemicals to determine exposure time effects, and the results are shown in Table 2. In all cases except 2,4-toluenediamine, EC₅₀⁵ (the EC₅₀ measured at 5 minutes) was equal to or greater than EC₅₀³⁰ (the EC₅₀ measured at 30 minutes). The greatest changes observed were cyanide, with decrease of EC₅₀ from 5.6 mg/l to 0.08 mg/l (see Figure 7) and cyclohexylamine, 4300 mg/l to 120 mg/l (see Figure 8) for EC₅₀⁵ and EC₅₀³⁰ respectively. These changes may

reflect time for either lethal accumulation or kinetic effects on enzyme systems of the bacteria. In the case of 2,4-toluenediamine, the increase in EC_{50} at longer exposure times may be due to a stimulation/excitation chemical effect on the bacteria. Of the fourteen chemicals tested, eight showed no or insignificant (<1%) change in EC_{50} with time, five showed greater toxicity, and one showed lesser toxicity.

Comparison of EC_{50} to LC_{50}

EC_{50} measurements have been compared to LC_{50} values for concentration relationships and hierarchy of toxicity. Table 1 lists reported MicrotoxTM and observed fish assay results (2). Table 3 shows the results of the chemicals tested in this study for the range of LC_{50} values. The general trends observed are that for the highly toxic chemicals, LC_{50} and EC_{50} values show good correlations in most cases. As LC_{50} toxicity decreases, EC_{50} toxicity also decreases, with EC_{50} being less conservative an indicator of toxic concentrations with decreasing toxicity.

Tables 5, 6 and 7 compare EC_{50} with specific fish species (fathead minnow, golden orfe, and bluegill sunfish, respectively) LC_{50} toxicity. EC_{50} is generally a good indicator of higher toxicities for all three fish species, and becomes less conservative with increasing EC_{50} concentrations. There are notable exceptions in each fish species for low LC_{50} concentrations. Propionitrile gave an EC_{50} of 5200 mg/l and an LC_{50} of 10 mg/l for fathead minnows. Similarly, TNT had an EC_{50} of 20 mg/l and an LC_{50} of 2.6 for the same species. The EC_{50} of allylamine measured 12500 mg/l and the LC_{50} for that compound was 70 mg/l for the

golden orfe. EC₅₀ results showed an order of magnitude difference higher LC₅₀ values for five of eight compounds in bluegill sunfish toxicity tests.

Mathematical relationships were formulated from literature LC₅₀ data, and Beckman literature and APCI generated EC₅₀ data. An exponential model of the form

$$LC_{50} = K(EC_{50})^n$$

yielded the model results for K and n shown in Table 4.

TABLE 4

<u>Species</u>	<u>K</u>	<u>n</u>	<u>Linear Correlation Coefficient</u>
Fathead Minnow	1.9	0.72	0.85
Bluegill Sunfish	0.52	0.77	0.85
Golden Orfe	39.8	0.30	0.61

Corresponding linearized plots of the data and fitted model are shown in Figures 4, 5, and 6, respectively. The model results are best viewed as preliminary, based on the small data sets available for EC₅₀. Additionally, the assumption of a direct relationship between chemical species toxicity for simple bacteria and macrovertebrates is simplistic. However, the models, in reality the data, show that EC₅₀ gives a useful indication of higher toxicity chemical species, i.e. low EC₅₀ values correspond to

low LC_{50} values. As the toxicity data bases increase, this hypothesis will be further tested, the model refined, and better relationships developed.

SUMMARY AND CONCLUSIONS

- o The Beckman MicrotoxTM is a quick and effective method for toxicity screening. MixrotoxTM compares favorably with other aquatic toxicity tests for relative toxicity determinations, i.e. the general order of toxicity is the same for LC_{50} and EC_{50} results.
- o The use of a toxicity standard is necessary for valid testing results.
- o Repeatability of MicrotoxTM testing is excellent. Although results indicate the need is not absolute, duplicate testing is recommended because of the ease and quickness of the MicrotoxTM procedure.
- o A preliminary exponential relationship for specific fish test toxicity (LC_{50}) and MicrotoxTM toxicity (EC_{50}) has been developed. This first-pass model agrees well for more highly toxic chemicals, with EC_{50} becoming a more liberal estimator for less toxic substances.

TABLE 1

SAMPLE COMPARISON OF SPECIFIC TOXICANTS (2)
(all results in mg/L)

<u>Toxicant</u>	<u>Microtox</u> (5 min EC ₅₀)	<u>Fish Assay</u> (96 hr LC ₅₀ *)
Mercury (II)	0.065	0.01 - 0.9
Pentachlorophenate	0.5	0.21 - 0.6
Aroclor 1240	0.7	0.3 - 1.0
p-Cresol	1.5	3.5 - 19
Ammonia (free)	2.0	0.068- 8.2
Benzene	2.0	17 - 32
Sodium Lauryl Sulfate	2.5	5 - 46
Zinc (II)	2.5	0.24 - 7.2
Malathion	3.0	0.07 - 19.5
Formaldehyde	3.0	18 - 185
Copper (II)	8.0	0.1 - 10.7
Cyanide as HCN	8.5	0.1 - 0.44
Trinitrotoluene	20	2.6
Phenol	25	9 - 66
Chromium VI	70	29 - 133
1-Butanol	3,300	1,200- 1,940
Isopropanol	4,200	4,200- 11,130
Urea	24,000	12,000
Ethanol	31,000	13,500

*LC₅₀: The concentration of a substance which is lethal to 50% of the test group.

Note: LC₅₀ data ranges were compiled using averages of testing performed using the following aquatic species:

Fathead Minnow	Goldfish
Bluegill Sunfish	Guppy
Rainbow Trout	Striped Bass
Water Flea	Golden Orfe
Daphnia	Bay Shrimp
Channel Catfish	

TABLE 2

AVERAGE EC₅₀* DATA
(mg/L)

<u>Compound</u>	<u>5 minutes</u>	<u>30 minutes</u>
Cyanide ^a	5.6	0.08
4,6-Dinitroorthocresol ^a	6.3	1.5
Ammonia	2.0	2.0
2,4-Dinitrophenol ^a	6.1	6.1
2,4-Dinitrotoluene ^a	33	21
2,4-Toluenediamine	73	86
Cyclohexylamine	4,200	120
Picric Acid	535	535
Propionitrile	5,200	5,200
Allylamine	13,000	12,500
n-Butylamine	18,500	18,500
Acetonitrile	24,000	24,000
Ethylamine	31,350	31,000
Diethylamine	38,000	32,000

*: EC₅₀ is an average of replicate testing.

a: Denotes a compound which EPA has listed as one of the 129 priority pollutants.

TABLE 3

Comparison of Relative Toxicity Using LC₅₀ and EC₅₀ Data

<u>Compound</u>	<u>LC₅₀ (96 hr)</u>	<u>EC₅₀ (5 min)</u>	<u>EC₅₀ (30 min)</u>
4,6-Dinitro-o-cresol	0.003 - 2.0	63	15
Ammonia	0.068 - 0.358	2.0	2.0
Picric Acid	0.09 - 0.167	535	535
Cyanide	0.1 - 0.3	5.6	0.08
2,4-Dinitrophenol	0.41 - 0.62	6.1	6.1
2,4-Toluenediamine	8 - 20	73	86
Propionitrile	10 - 30	5200	5200
Cyclohexylamine	58 - 195	4200	120
Allylamine	6 - 76	13000	12500
n-Butylamine	171	18500	18500
Ethylamine	40 - 240	31500	31000
Diethylamine	850	38000	32000
Acetonitrile	1000 - 7050	24000	24000

TABLE 5

EC₅₀ Toxicity Results Comparison to Fathead Minnow LC₅₀ Assays

<u>Compound</u>	LC ₅₀ (3)	EC ₅₀
Cyanide	0.12	0.08
Aroclor 1240	0.3	0.7
p-Cresol	20	1.5
Ammonia	1.6 - 2.0	2.0
Benzene	30	2.0
Malathion	9.0	3.0
Phenol	29	25
Trinitrotoluene	2.6	20
1-Butanol	1900	3300
Isopropanol	11000	4200
Propionitrile	10	5200
Acetonitrile	1000	24000
Ethanol	13500	31000

TABLE 6

EC₅₀ Toxicity Results Comparison to Golden Orfe LC₅₀ Assays

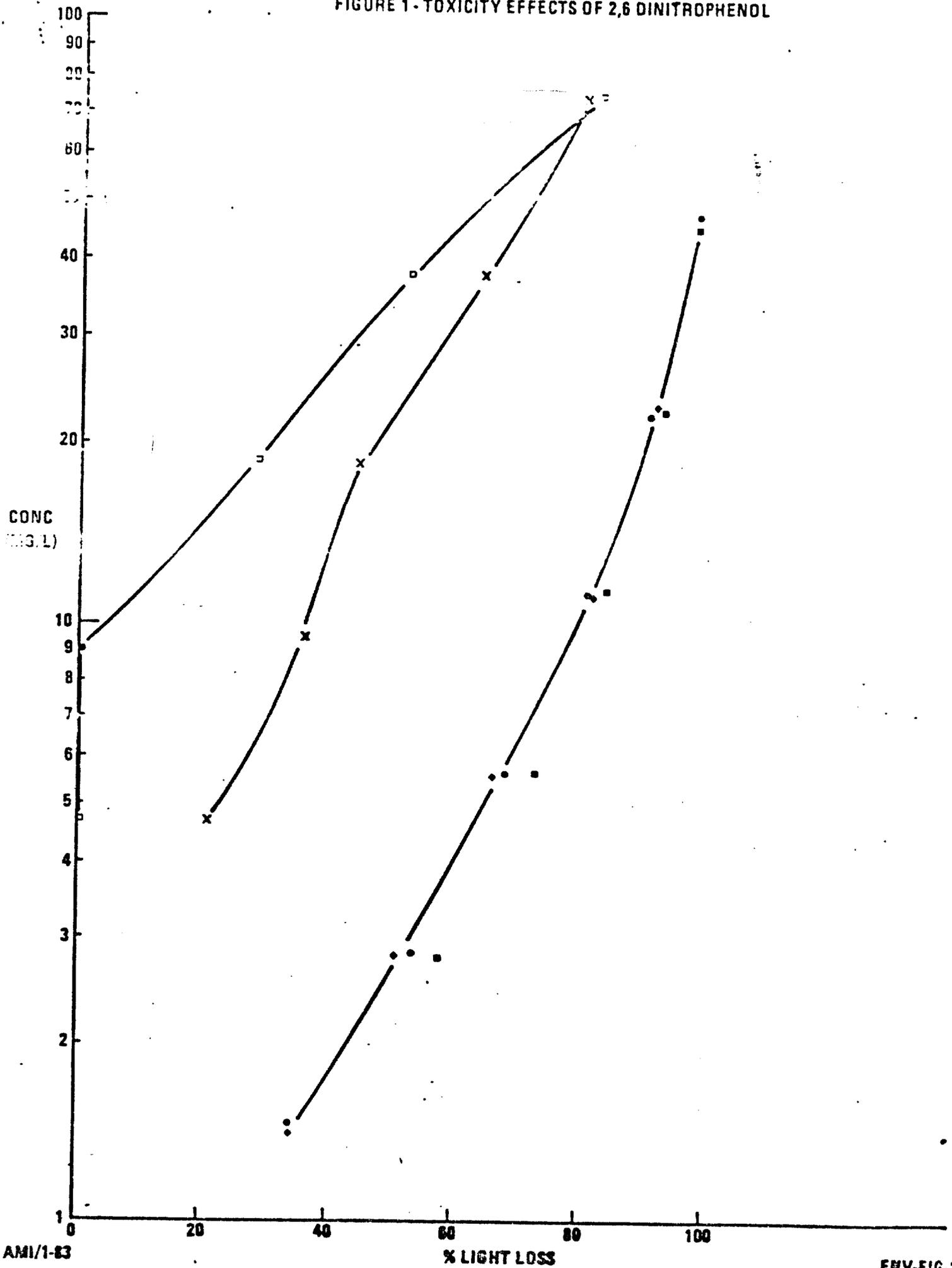
<u>Golden Orfe</u>	LC ₅₀ (3)	EC ₅₀
Benzene	33/45/62	2.0
Formaldehyde	75	3.0
Cyclohexylamine	130	120
n-Butylamine	170	1850
1-Butanol	1200	3300
Allylamine	70	12500
Acetonitrile	6500	24000
Ethylamine	240	31000
Ethanol	4000	31000

TABLE 7

EC₅₀ Toxicity Results Comparison to Bluegill Sunfish LC₅₀ Assays

<u>Compound</u>	LC ₅₀ (3)	EC ₅₀
Cyanide	0.20	0.08
Aroclor 1240	0.984	0.7
Benzene	22	2.0
Malathion	0.11	3.0
2,4-Dinitrophenol	0.62	6.1
Phenol	26	25
2,4-Toluenediamine	8	100
Acetonitrile	1850	24000

FIGURE 1 - TOXICITY EFFECTS OF 2,6 DINITROPHENOL



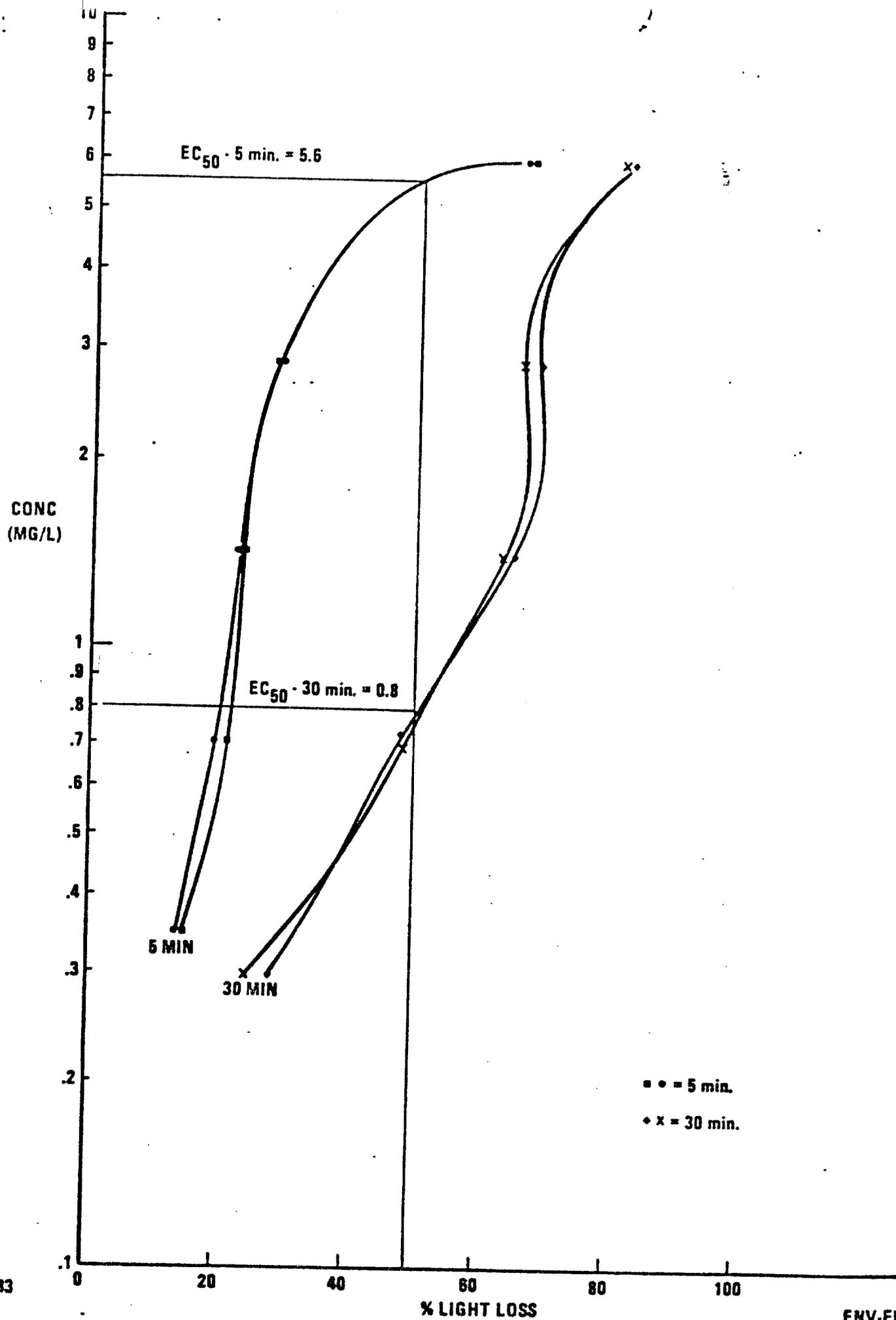


FIGURE 3 - TOXICITY EFFECTS OF 2,4 TOLUENEDIAMINE

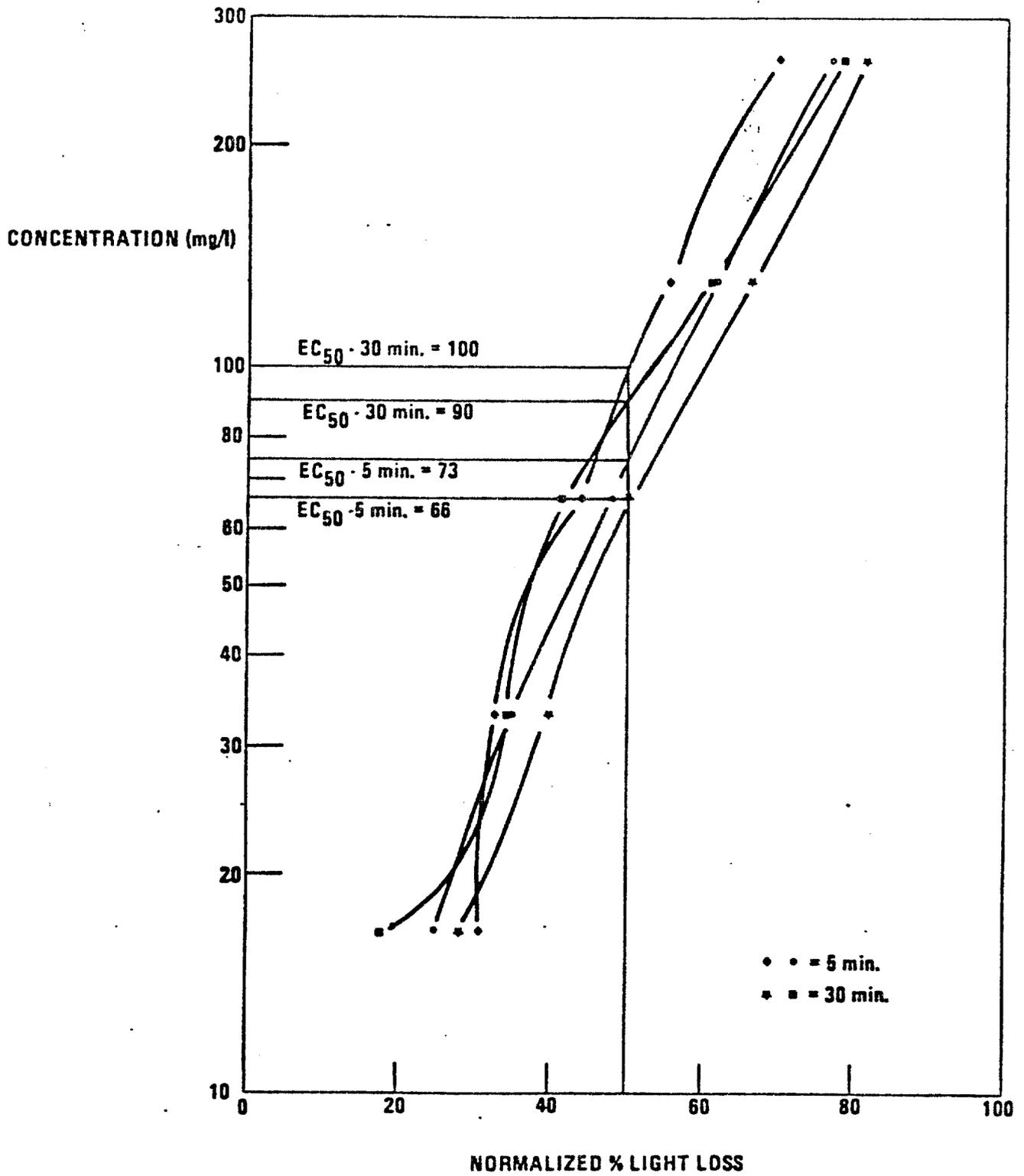


FIGURE 4 - COMPARISON OF EC₅₀ TO FATHEAD MINNOW LC₅₀

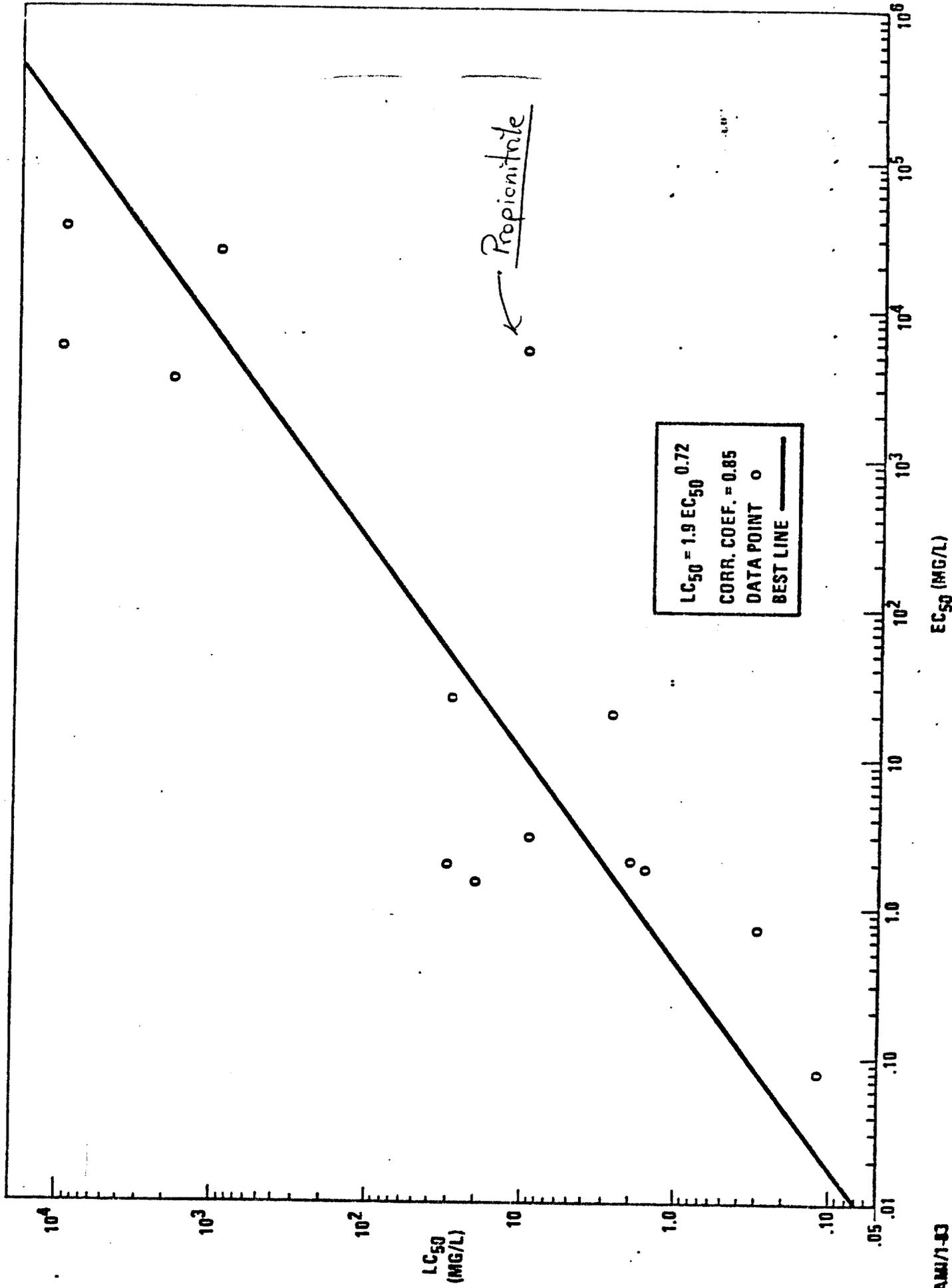
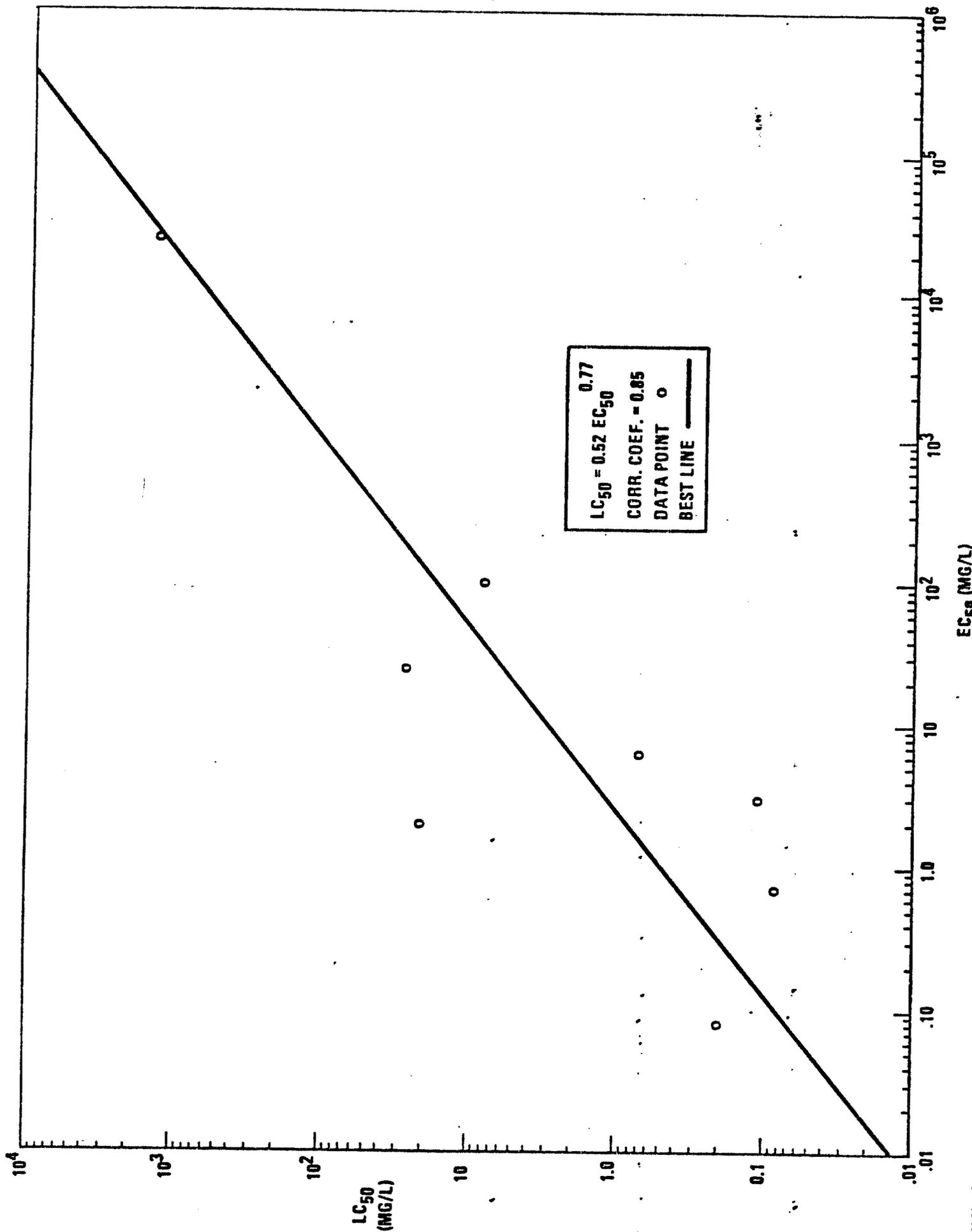


FIGURE 3 COMPARISON OF EC₅₀ TO BLUEGILL SUNFISH LC₅₀



50 50 50

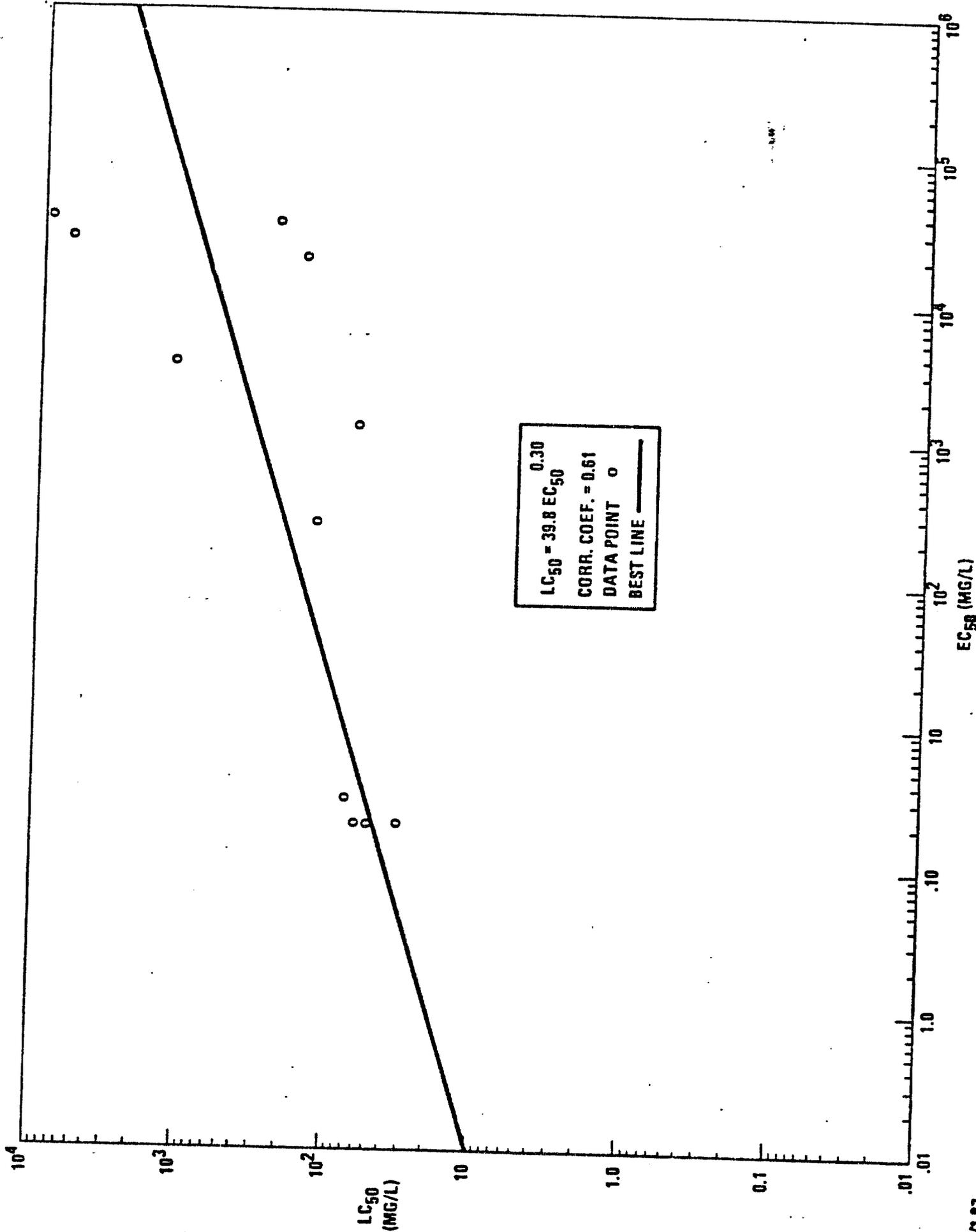


FIGURE 7 - TOXICITY EFFECTS OF CYANIDE

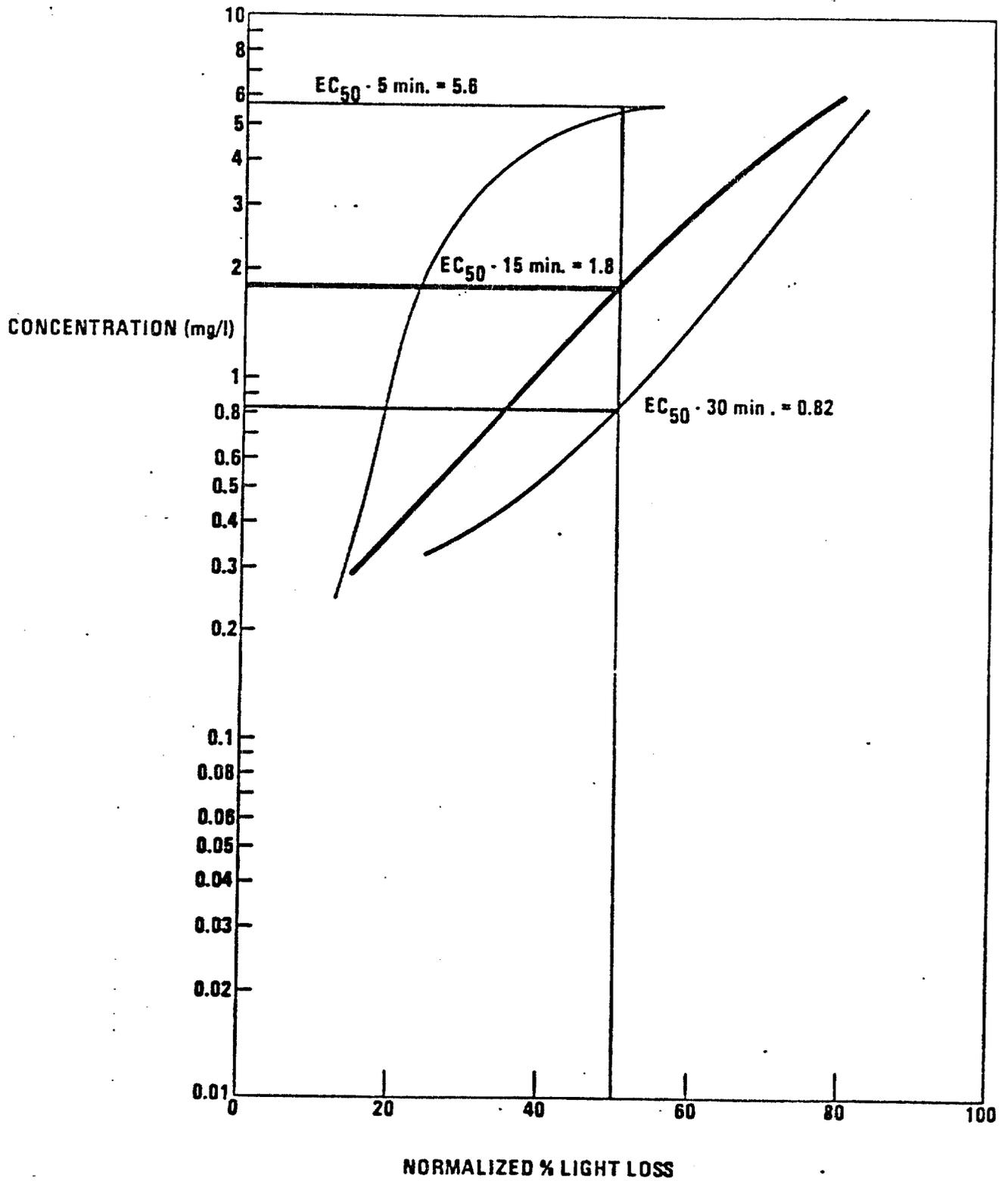
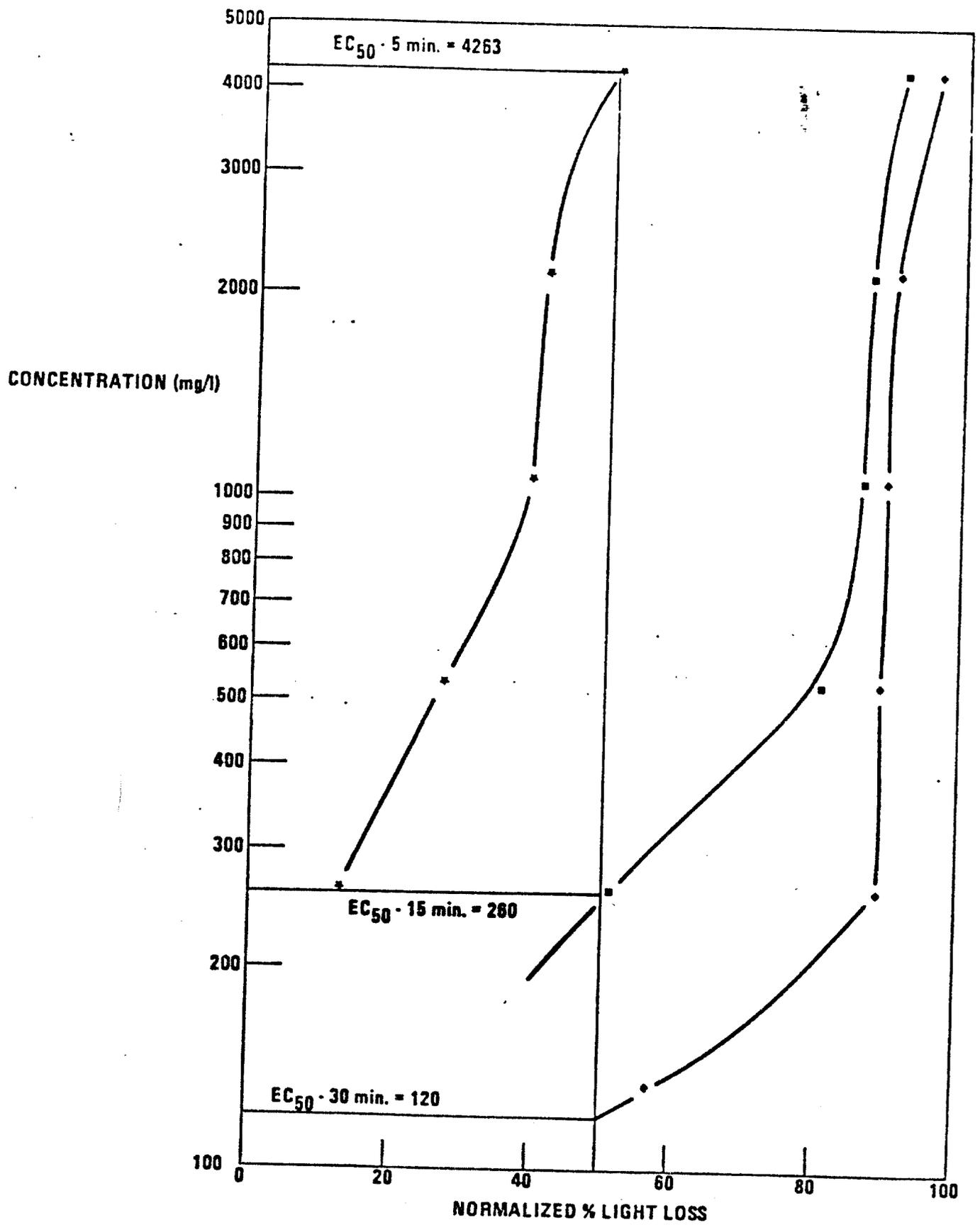


FIGURE 8 · TOXICITY EFFECTS OF CYCLOHEXYLAMINE



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