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Document Title	SUPPORT: ACUTE TOXICITY OF ALLYL-METHACRYLATE TO FATHEAD MINNOW (PIMEPHALES PROMELAS), WITH COVER LETTER DATED 6/4/2001		
Chemical Category	2-PROPENOIC ACID, 2-METHYL-, 2-PROPENYL ESTER		

degussa.

8EHQ-0601-14632

Management Services

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June 4, 2001

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U.S. Environmental Protection Agency
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Washington, DC 20460

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ATTN: Section 8 (e) Coordinator

RE: Product Name: Allyl Methacrylate
CAS Registry No. 96-05-9
CAS Registry Name: 2-Propenoic acid, 2-methyl-, 2-propenyl ester

Dear Sir or Madam:

Degussa Corporation has received from Röhm GmbH, the enclosed report on "Acute Toxicity of Allyl-methacrylate to Fathead Minnow (*Pimephales promelas*)," which studies were conducted by Fraunhofer-Institut für Umweltchemie and Ökotoxikologie. This is a follow up to the TSCA 8(e) submission made December 22, 1999 on the subject product.

Pursuant to Section 8 (e) of the Toxic Substances Control Act, Degussa Corporation provides this information to EPA.

Sincerely,

Kisha Pippins
Product Safety Specialist



8EHQ-99-14632

cc: R. Brazicki



89010000222

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Fraunhofer Institut
Umweltchemie und
Ökotoxikologie

TEST REPORT:	Fathead Minnow; ACUTE TOXICITY
TEST SUBSTANCE:	Allyl-methacrylate
GLP CODE:	RÖH-019/4-13

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TEST REPORT

**Acute Toxicity of
Allyl-methacrylate
to Fathead Minnow (*Pimephales promelas*)**

Date: May 17, 2001

Distribution of test reports:

Sponsor (original)

GLP- Archive (original)

Sponsor (2 copies)

GLP- archive (copy)

Study director (copy)

6215-3-03



TEST REPORT: Fathead Minnow; ACUTE TOXICITY
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TEST REPORT

- 1 **Test**

Fathead Minnow (*Pimephales promelas*), Acute Toxicity Test, flow-through-conditions (EC dir. 92/69/EEC (1))

Test substance: Allyl-methacrylate

GLP-Code: RÖH-019/4-13

- 2 **Sponsor**

RÖHM GmbH & Co. KG, Chemische Fabrik
Kirschenallee
D-64293 Darmstadt

Monitor: Dr. H. Müllerschön

- 3 **Testing facility**

Fraunhofer-Institut für
Umweltchemie und Ökotoxikologie
Postfach 1260
D-57377 Schmallenberg-Grafschaft

Management: Prof. Dr. W. Klein

Study director: Dr. A. Schmitz
Deputy: Dr. C. Schäfers

Chemical investigator: Dr. J. Müller

Technical staff: U. Boshof
W. Böhmer
J. Nowak

Quality Assurance Unit:
Dr. U. Fritsche
Dr. G. Wasmus



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4 Summary

At the Fraunhofer Institut für Umweltchemie und Ökotoxikologie the toxic effects of the test substance Allyl-methacrylate on aquatic secondary consumers were examined. The study was sponsored by RÖHM GmbH, Darmstadt.

Pimephales promelas (Fathead Minnow) was chosen as test organism. The acute mortality under flow-through conditions was assessed at nominal test concentrations of 0.34; 0.75; 1.65; 3.64; 8.00 mg*l⁻¹ (test 1) and 0.80; 1.00; 1.20 mg*l⁻¹ (test 2) after 24, 48, 72 und 96 hours. The test was performed in accordance with the EC dir. 92/69/EEC (1).

The effect concentrations refer to the mean measured concentrations, because the measured concentrations in test 1 deviated by more than 20 % from the nominal values.

Lethal effect concentrations (mg*l⁻¹) of Allyl-methacrylate after 96 h

LC10	LC50	95 % confidence limits of LC50
0.41	0.61	0.53 - 0.71

The determination of NOEC and LOEC of Allyl-methacrylate based on the effect mortality resulted in:

NOEC: 0.17 mg*l⁻¹
LOEC: 0.52 mg*l⁻¹



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5 Test substance

- 5.1 Specification
- 5.1.1 Trade name: Allyl methacrylate
- 5.1.2 Chemical name: 2- Propenoic acid, 2-methyl-,2-propenyl ester
- 5.1.3 CAS-Number: 96-05-9
- 5.1.4 Lot Number / Batch Number: 32063708 / 1290710258
- 5.1.5 Purity: 98.60 %
- 5.1.6 Water solubility: 4.0 g*l⁻¹
- 5.1.7 Chemical stability (Water/Light): Thermically stable
- 5.1.8 Specific density (20 °C): 0.94 g/cm³ at 20 °C
- 5.1.9 State of matter and appearance: Colourless liquid, pungent odour
- 5.1.10 Safety data sheet: Available
- 5.1.11 Expiry date: 6 months from date of delivery
- 5.1.12 Origin of the test substance: Sponsor
- 5.2 The identity and purity of the test substance were not confirmed analytically by the testing facility.

6 GLP

The test was performed in accordance with the Principles of Good Laboratory Practice (2).



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7 Test principle

Fathead Minnow (*Pimephales promelas*) were exposed in two independent studies to different concentrations of the test substance under flow-through conditions for a period of 96h. The mortality was determined after 24h, 48h, 72h and 96h. The test was performed in accordance with the EC dir. 92/69/EEC (1).

8 Details concerning the test

8.1 Characterization of the test organism

8.1.1 Justification for the use of the test organism:

Pimephales promelas (Fathead Minnow) is recommended by the EC-experts (1) as test organism representing aquatic vertebrates.

8.1.2 Test organism:

Specification: *Pimephales promelas* (Teleostei, Cyprinidae)

Source: BIO INTERNATIONAL B.V.
P.O. Box 616
NL-6040 AP Roermond
Netherlands

Holding: The fish were acclimatized in water of the same quality as used in the test. They were fed daily with TetraMin^R Hauptfutter (Tetra Werke, Melle, Germany).

Health: The criteria of the test guideline (1) were considered. Only healthy fish without diseases and abnormalities were used in the study.

8.2 Dilution water

Purified drinking water was used as dilution water. The purification included filtration with charcoal, aeration and passage through a lime stone column. The water was aerated to oxygen saturation prior to preparation of the test solutions.

8.3 Test procedure

The fish were exposed under flow-through conditions to various concentrations of the test substance. The stock solutions were prepared freshly every day. The temperature during the test was set to 23.0 °C. Mortality and abnormal behavior was recorded after 24h, 48h, 72h and 96h. Dead animals were eliminated from the vessels as soon as they were discovered.



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15 l glass aquaria were used as test vessels and filled with 10 l of test solution. No aeration of the solutions was necessary, because the oxygen saturation in all test vessels was higher than 60 %. 10 fish were used for each test concentration and for the controls (dilution water). The light/dark cycle was adjusted to 14/10 hours. No feeding occurred during the test as well as 24 h before test start. An aliquot of 30 fish was used to measure the fish length and weight at the beginning of the study.

pH value (pH-Meter, WTW 535), oxygen concentration (WTW Digital-Sauerstoff-Meßgerät Oxi 196), and temperature (Digitalthermometer, Roth) were measured directly before adding the fish and afterwards once per day.

Because the LC50 value calculated from the first test (referred to as test 1) could only be derived from fitting a curve through values of very low and very high mortality with a factor of 2.2 between the respective nominal concentrations, a second test (referred to as test 2) was performed in order to test concentrations closer to the LC50.

Test concentrations: The following nominal concentrations were prepared:
0 (control); 0.34; 0.75; 1.65; 3.64; 8.00 mg·l⁻¹ (test 1)
0 (control); 0.80; 1.00; 1.20 mg·l⁻¹ (test 2)

Preparation of the test substance:

The pure test substance was weighed in directly to prepare five (three) stock solutions which were filled into glass syringes and were applied by dosage pumps to a continuous dilution water flow in order to prepare the test medium in the test vessels. The flow through rate was 2.5 l · h⁻¹. Purified drinking water was tested as control.

8.4 Chemical analysis

The test concentrations were checked analytically by HPLC at test start and every 24 hours. The analytical measurement method by HPLC was performed according to a study specific work instruction (amendment to the protocol, raw data).

Hardware-system: GYNKOTHEK M 480 (pump); GYNKOTHEK Gin 3 160 (injector);
GYNKOTHEK UVD 160S7320S (UV-detector).
Grasping of dates: PC AT486 with GYNKOSOFT version 5.50
Column: 250 mm * 4.6 mm; VDS optilab ODS Hypersil; 5 µm
Mobile Phase: 60 % acetonitrile, 40 % pure water
Temperature: room temperature
Detection: UV at 200 nm
Injection volume: 50 µl
Calibration interval: 0.19 - 9.4 mg/l (test 1) ; 0.094 - 1.57 mg/l (test 2)



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8.5 Evaluation

The NOEC and the LOEC were determined directly in the acute test. The LC50 and LC10 values as well as the confidence limits of the LC50 were derived from a concentration-effect curve fitted to the data by non-linear regression (probit analysis (3)).

9 Test conditions

9.1 Fish size at test start

From the stock of fish of which individuals were taken out for testing, three samples of 10 fish each were taken of which lengths and weights were measured.

The results of the representative length determination (Tables 1, 2) indicate, that the fish lengths (3.6 – 4.6 cm and 4.6 – 5.8, respectively) were within the range of sizes recommended by the EC directive (1) of 5 ± 2 cm.

Table 1: Fish length (cm) at start of test 1
(three samples of 10 fish each were measured)

Fish No.	Sample 1 (cm)	Sample 2 (cm)	Sample 3 (cm)
1	4.2	4.2	4.2
2	4.3	4.3	3.6
3	4.0	4.2	4.6
4	4.2	4.5	4.6
5	4.2	4.2	4.0
6	4.3	4.5	4.6
7	4.0	4.4	3.9
8	4.1	4.0	3.9
9	3.7	4.3	4.1
10	3.9	4.2	4.0
min. value	3.7	4.0	3.6
max. value	4.3	4.5	4.6
mean (n = 30)		4.3	
standard deviation		0.3	



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Table 2: Fish length (cm) at start of test 2
(three samples of 10 fish each were measured)

Fish No.	Sample 1 (cm)	Sample 2 (cm)	Sample 3 (cm)
1	4.8	5.5	5.0
2	5.3	4.8	5.2
3	5.1	5.4	5.0
4	4.7	5.8	5.5
5	5.3	4.6	5.0
6	5.0	4.6	5.3
7	5.4	4.6	4.6
8	4.7	5.2	4.8
9	4.7	4.7	5.1
10	5.1	4.6	5.6
min. value	4.7	4.6	4.6
max. value	5.4	5.8	5.6
mean (n = 30)	5.0		
standard deviation	0.3		

The results of the representative weight determination (Tables 3, 4) indicate, that with a mean weight of 0.67 g and 1.07 g, respectively, 10 individuals per vessel and a volume of the test solution of 10 l, the average loadings in the flow-through tests were about 0.7 g*l⁻¹ and 1.1 g*l⁻¹, respectively.

Table 3: Fish weight (g) at start of test 1 (three samples of 10 fish each were weighed)

Fish No.	Sample 1 (g)	Sample 2 (g)	Sample 3 (g)
1	0.75	0.64	0.67
2	0.77	0.79	0.48
3	0.69	0.69	0.85
4	0.68	0.94	0.96
5	0.63	0.75	0.45
6	0.57	0.77	0.84
7	0.62	0.68	0.54
8	0.61	0.67	0.49
9	0.51	0.81	0.71
10	0.57	0.63	0.55
min. value	0.51	0.63	0.45
max. value	0.77	0.94	0.96
mean value (n=30)	0.67		
standard deviation	0.12		



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Table 4: Fish weight (g) at start of test 2 (three samples of 10 fish each were weighed)

Fish No.	Sample 1 (g)	Sample 2 (g)	Sample 3 (g)
1	0.84	1.29	1.15
2	1.30	0.95	1.35
3	1.06	1.23	1.17
4	0.97	1.62	1.21
5	1.20	0.80	1.06
6	1.05	0.67	1.09
7	1.28	0.71	0.79
8	0.78	1.11	0.93
9	0.98	1.01	0.90
10	1.06	0.91	1.55
min. value	0.78	0.67	0.79
max. value	1.30	1.62	1.55
mean value (n=30)	1.07		
standard deviation	0.23		

9.2 Water quality parameter values throughout the test

The pH value of the test solutions during test 1 and 2 varied between 7.6 and 8.8 (Tables 5). The oxygen saturation in all test vessels was between 88 % and 98 %. In the test the temperature was in the range of 22.9 and 23.3 °C (Table 6).

Table 5: pH-values throughout test 1 and test 2

Hour of the test	nominal concentrations (mg·l ⁻¹)									
	test 1						test 2			
	control	0.34	0.75	1.64	3.64	8.00	control	0.80	1.00	1.20
0	8.2	8.2	8.2	8.3	8.2	8.3	8.0	8.0	8.0	8.1
24	8.4	8.8	8.5	8.5	8.5	8.5	7.9	8.3	8.4	8.4
48	8.4	8.5	8.4	8.5	n.d.	n.d.	7.6	8.2	8.1	8.3
72	8.4	8.7	8.5	8.7	n.d.	n.d.	7.7	8.2	8.2	n.d.
96	8.4	8.4	8.3	8.5	n.d.	n.d.	8.1	8.3	8.3	n.d.
Min. value	8.2	8.2	8.2	8.3	8.2	8.3	7.6	8.0	8.0	8.1
Max. value	8.4	8.8	8.5	8.7	8.5	8.5	8.1	8.3	8.4	8.4

n.d. = not determined (all fish dead)



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Table 6: Oxygen concentrations ($\text{mg}\cdot\text{l}^{-1}$) and temperature during test 1 and test 2

Hour of the test nominal conc. ($\text{mg}\cdot\text{l}^{-1}$)	0	24	48	72	96
Test 1: control	8.0	8.0	7.9	8.4	8.3
0.34	8.4	7.6	8.0	8.1	7.5
0.75	8.1	7.4	8.0	8.1	7.9
1.64	8.0	7.5	8.2	8.2	8.0
3.64	8.1	7.6	n.d.	n.d.	n.d.
8.00	8.0	7.5	n.d.	n.d.	n.d.
min. value	8.0	7.4	7.9	8.1	7.5
max. value	8.4	8.0	8.2	8.4	8.3
oxygen saturation value	8.6	8.6	8.5	8.7	8.6
temperature ($^{\circ}\text{C}$)*	23.2	23.3	23.2	22.9	23.1
Test 2: control	8.5	7.3	6.3	7.0	7.8
0.80	8.4	8.4	8.3	8.5	7.8
1.00	8.4	8.0	8.0	8.4	8.3
1.20	8.3	8.4	8.3	n.d.	n.d.
min. value	8.3	7.3	6.3	7.0	7.8
max. value	8.5	8.4	8.3	8.5	8.3
oxygen saturation value	7.9	8.2	8.4	8.3	8.4
temperature ($^{\circ}\text{C}$)*	23.1	23.0	23.2	23.0	23.1

n.d. = not determined (all fish dead); * single measurement in a water bath holding all test vessels



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10 Results

10.1 Test concentrations

The nominal test concentrations as well as the measured concentrations of the test substance in tests 1 and 2 are listed in table 7. The measured values deviated by more than 20 % from nominal values at all dose levels of test 1. Thus, mean measured concentrations were taken as basis for the calculation of the lethal effect concentrations.

Table 7: Test concentrations (mg·l⁻¹) - nominal and measured concentrations of the test substance

Nominal test concentrations	Measured test concentrations							Deviations (%) from nom. values
	0 h	6 h	24 h	48 h	72 h	96 h	Mean ± s.d.	
Controls	0.00	0.00	0.00	0.00	0.00	0.00	0.00 ± 0.00	
Test 1: 0.34	0.20	0.17	0.19	0.11	0.10	0.25	0.17 ± 0.06	- 50
Test 1: 0.75	0.46	0.72	0.62	0.40	0.56	0.56	0.52 ± 0.13	- 31
Test 2: 0.80	0.80	0.62	1.17	0.94 / 0.66	0.64	0.59	0.77 ± 0.21	- 4
Test 2: 1.00	0.87	0.71	1.04	0.76 / 0.69	0.84	0.86	0.82 ± 0.12	- 18
Test 2: 1.20	1.68	1.03	1.47	1.27	n.d.	n.d.	1.36 ± 0.26	+ 13
Test 1: 1.65	1.67	1.33	1.09	0.95	0.69	1.36	1.18 ± 0.33	- 29
Test 1: 3.64	2.78	2.21	2.76	n.d.	n.d.	n.d.	2.58 ± 0.29	- 29
Test 1: 8.00	5.55	3.80	4.15	n.d.	n.d.	n.d.	4.50 ± 0.83	- 44

s.d. = standard deviation; n.d. = not determined (all fish dead)

Table 8: Test concentrations (mg·l⁻¹) - mean measured concentrations of the test substance after different exposure periods (increasing number of measured samples)

Nominal test concentrations	Mean measured test concentrations after			
	24 h	48 h	72 h	96 h
Test 1: 0.34	0.18	0.15	0.15	0.17
Test 1: 0.75	0.60	0.55	0.51	0.52
Test 2: 0.80	0.86	0.83	0.80	0.77
Test 2: 1.00	0.87	0.81	0.82	0.82
Test 2: 1.20	1.39	1.36	1.36	1.36
Test 1: 1.65	1.36	1.26	1.14	1.18
Test 1: 3.64	2.58	2.58	2.58	2.58
Test 1: 8.00	4.50	4.50	4.50	4.50



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10.2 Mortality during the test

The cumulative mortalities of fish during the test period (Table 9) indicate a steep concentration-response relationship with close LC10 and LC50 values (Table 10, Figure 1).

Table 9: Cumulative mortality during the test period of 96 h.

Mean measured test concentrations (mg·l ⁻¹)	Cumulative mortality			
	24 h	48 h	72 h	96 h
0.00	0	0	0	0
0.17	0	0	0	0
0.52	0	1	1	2
0.77	0	0	4	5
0.82	0	3	7	9
1.18	0	9	9	9
1.36	0	10	10	10
2.58	10	10	10	10
4.50	10	10	10	10

Table 10: Lethal effect concentrations (mg·l⁻¹) of Allyl-methacrylate. C.I.: confidence limits (based on mean measured concentrations given in table 8)

Hours of test	NOEC	LOEC	LC10	LC50	C.I. of LC50
24	1.36	2.58	1.68	1.87	0.74 – 4.72
48	0.17	0.52	0.54	0.89	0.76 – 1.03
72	0.17	0.52	0.42	0.64	0.55 – 0.75
96	0.17	0.52	0.41	0.61	0.53 – 0.71



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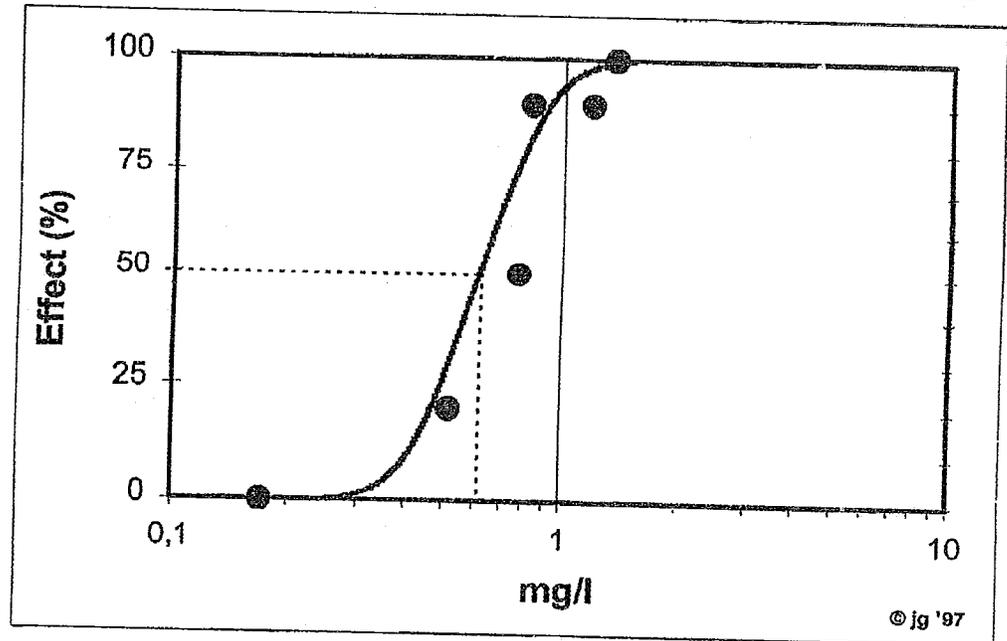


Figure1: Concentration-effect relationship (96 h)

11 Record keeping

The raw data of the study, one original and a copy of the study report and an aliquot of the test substance are kept in the archives of Fraunhofer-Instituts für Umweltchemie und Ökotoxikologie, D-57392 Schmallenberg-Grafschaft, FRG, according to (2).



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12 Statement of GLP-Compliance:

The study was conducted in compliance with Good Laboratory Practice Regulations (GLP). There were no deviations from legal regulations.

Schmallenberg-Grafschaft,

Date: *May 18, 2001* Management

W. Klein
(Prof. Dr. W. Klein)

Date: *May 17, 2001* Study Director
(Deputy)

C. Schäfers
(Dr. C. Schäfers)

Date: *May 17, 2001* Chemical investigator

J. Müller
(Dr. J. Müller)



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13 Quality assurance statements:

Title of the Study: Fish (Fathead Minnow)
Acute Toxicity (EC dir. 92/69/EEC)

Study-Code: RÖH-019/4-13

Test substance: Allyl methacrylate

The Quality Assurance Unit of the testing facility inspected the study and audited the final report according to GLP-Regulations.

Dates of QAU Inspections: October 19th, 1999: Test 1: Determination of pH value and oxygen concentration after 24 h.

January 14th, 2000: Test 2: Observation of the behaviour after 48 h.

The results reported in this study were checked on the basis of our current SOPs and to the best of our knowledge accurately reflect the raw data.

Schmallenberg-Grafschaft,

Date: May 17, 2001

M. Frietsche
QAU - Officer



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14 References

- (1) EU-Richtlinie 92/69/EWG Teil C: Methoden zur Bestimmung der Ökotoxizität. C.1. Akute Toxizität für Fische. Amtsblatt der Europäischen Gemeinschaften Nr. L 383 A/163 vom 29.12.1992.
- (2) Grundsätze der Guten Laborpraxis (Good Laboratory Practice, GLP). Gesetz zum Schutz vor gefährlichen Stoffen (ChemG), Anhang 1, Version from 25.07.1994, published in: BGBl. I No. 47 from 29.07.1994, last change: 14.05.1997 (BGBl. I No. 30 from 22.05.1997 p. 1060).
- (3) Finney, D.J.: Statistical Method in Biological Assay. 2nd ed., London. 1984



TEST REPORT: Fathead Minnow; ACUTE TOXICITY
TEST SUBSTANCE: Allyl-methacrylate
GLP CODE: RÖH-019/4-13

15 GLP-certificate



Ministerium für Umwelt, Raumordnung und Landwirtschaft
des Landes Nordrhein-Westfalen

Postanschrift: 40190 Düsseldorf

Aktenzeichen: IV C 4 - 31.11 79.04

GLP-Bescheinigung

Bescheinigung

Hiermit wird bestätigt, daß die Prüfeinrichtung
Fraunhofer-Institut für Umweltchemie und
Ökotoxikologie (IUCT)
Auf dem Aberg 1

in 57392 Schmallenberg
(Ort, Anschrift)

Der W.O.
(Firma)

am 12. August 1998
(Datum)

von der für die Überwachung zuständigen
Behörde über die Einhaltung der Grundsätze
der Guten Laborpraxis inspiziert worden ist.

Es wird hiermit bestätigt, daß folgende Prüfungen
in dieser Prüfeinrichtung nach den Grundsätzen
der Guten Laborpraxis durchgeführt wurden.

Certificate

It is hereby certified that the test facility
Fraunhofer-Institut für Umweltchemie und
Ökotoxikologie (IUCT)
Auf dem Aberg 1

in 57392 Schmallenberg
(location, address)

of W.O.
(company name)

on 12. August 1998
(date)

was (were) inspected by the competent authority
regarding compliance with the Principles of
Good Laboratory Practice.

It is hereby certified that following studies in this
test facility are conducted in compliance with the



TEST REPORT: Fathead Minnow; ACUTE TOXICITY
TEST SUBSTANCE: Allyl-methacrylate
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Kategorie 1
Prüfungen zur Bestimmung der physikalisch-
chemischen Eigenschaften und Gehaltsbestim-
mungen

category 1
physical-chemical testing

Kategorie 4
Ökotoxikologische Prüfungen zur Bestimmung
der Auswirkungen auf aquatische und
terrestrische Organismen

category 4
environmental toxicity studies on aquatic and
terrestrial organism

Kategorie 5
Prüfungen zum Verhalten im Boden, im Wasser
und in der Luft; Prüfungen zur Bioakkumulation
und zur Metabolisierung

category 5
studies on behaviour in water, soil and air;
bioaccumulation

Kategorie 6
Prüfungen zur Bestimmung von Rückstän

category 6
residue studies

Kategorie 7
Prüfungen zur Bestimmung der Auswirkungen
auf Mesokosmen und natürliche Ökosysteme

category 7
studies on effects on mesocosms and natural
ecosystems

Kategorie 9: Sonstige Prüfungen
Modell- und Simulationsrechnungen für das
Verhalten von Stoffen in der Umwelt

category 9: other studies, specify
Model and simulation based calculation of
environmental behaviour of chemicals

Düsseldorf, 12. Mai 1999

Im Auftrag

(Dr. Harald Friedrich)



(official-seal)