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10F0449/895178
GOLDEN ORFE
(LEUCISCUS IDUS L., GOLDEN VARIETY)

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BASF AKTIENGESELLSCHAFT
DEPARTMENT OF TOXICOLOGY

REPORT ON THE STUDY OF THE ACUTE TOXICITY

NAME OF TEST SUBSTANCE: DIBUTYLPHTHALAT

ANIMAL SPECIES: GOLDEN ORFE (LEUCISCUS IDUS L., GOLDEN VARIETY)

PROJECT NO.: 10F0449/895178

SPONSOR: BASF AKTIENGESELLSCHAFT

SUMMARY AND EVALUATION:

LC 50 (MG/L; NOMINAL CONCENTRATIONS) AFTER

1H	GREATER	10	(MG/L)	(1% SIGNIFICANCE LEVEL)
4H	GREATER	10	(MG/L)	(1% SIGNIFICANCE LEVEL)
24H	GREATER	4.6	(MG/L)	(1% SIGNIFICANCE LEVEL)
	LOWER	10	(MG/L)	(1% SIGNIFICANCE LEVEL)
48H	GREATER	4.6	(MG/L)	(1% SIGNIFICANCE LEVEL)
	LOWER	10	(MG/L)	(1% SIGNIFICANCE LEVEL)
72H	GREATER	4.6	(MG/L)	(1% SIGNIFICANCE LEVEL)
	LOWER	10	(MG/L)	(1% SIGNIFICANCE LEVEL)
96H	GREATER	4.6	(MG/L)	(1% SIGNIFICANCE LEVEL)
	LOWER	10	(MG/L)	(1% SIGNIFICANCE LEVEL)

SYMPTOMS :

APATHY, TUMBLING

NO OBSERVED EFFECT CONCENTRATION: 2.15 MG/L

MAXIMUM CONCENTRATION TESTED CAUSING NO MORTALITY: 4.64 MG/L

MINIMUM CONCENTRATION TESTED CAUSING 100% MORTALITY: 10.0 MG/L

V. Kirsch
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HEAD OF SECTION

R. Munk
DR. RER. NAT. R. MUNK
STUDY DIRECTOR

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TEST SUBSTANCE:

TEST SUBSTANCE NO.: 89/449

NAME OF TEST SUBSTANCE: DIBUTYLPHTHALAT

BATCH NO.: P. 66A/89

DEGREE OF PURITY: > 99.0%

HOMOGENEITY: ENSURED SINCE THE TEST SUBSTANCE IS A GENUINE LIQUID

SOLUBILITY IN WATER: SLIGHTLY SOLUBLE ACCORDING TO SPONSOR
(CF. REMARKS P. 6)

PHYSICAL STATE: LIQUID

CHARACTERIZATION: DETAILS ON THE CHARACTERIZATION ARE INCLUDED
IN THE RAW DATA AND MAY BE REQUESTED FROM THE
SPONSOR.

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METHOD:

THE METHOD USED CLOSELY FOLLOWED THE GUIDELINE OF DIN 38 412
"TESTVERFAHREN MIT WASSERORGANISMEN (GRUPPE L). ALLGEMEINE HINWEISE
ZUR PLANUNG, DURCHFUEHRUNG UND AUSWERTUNG BIOLOGISCHER TEST-
VERFAHREN (L1)" UND "BESTIMMUNG DER WIRKUNG VON WASSERINHALTSSTOFFEN
AUF FISCH - FISCHTEST (L15)", JUNE 1982, USING A STATIC PROCEDURE.

PHOTOPERIOD: 16 HOURS LIGHT AND 8 HOURS DARKNESS

REASONS FOR THE SELECTION OF THE CONCENTRATIONS:
BASED ON THE RESULTS OF A RANGE FINDING STUDY
(LC 50 AFTER 96 H: < 10 MG/L) THE CONCENTRATIONS,
SPACED BY A FACTOR OF ABOUT 2.2, WERE FIXED AS FOLLOWS:
0.1, 0.215, 0.464, 1.0, 2.15, 4.64 AND 10 MG/L.

AERATION: SLIGHT

PREPARATION OF THE TEST SUBSTANCE: THE PRODUCT DISSOLVED IN ACETONE
(1.0 G/100 ML) WAS ADDED TO THE TEST WATER;
SUBSEQUENTLY THE FISH WERE PLACED INTO THE
AQUARIA. ANALOGOUSLY TO THE HIGHEST AMOUNT
OF SOLVENT USED, 1 ML ACETONE / LITER OF
TEST WATER WAS ADDED TO A CONTROL AQUARIUM.

RETENTION OF RECORDS:

THE RAW DATA AND THE ORIGINAL OF THE REPORT ARE STORED IN THE
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TEST ANIMALS:

ANIMAL SPECIES: GOLDEN ORFE (LEUCISCUS IDUS L., GOLDEN VARIETY)

CONSIGNMENT DATED: MAY 10, 1989

SUPPLIER: FISCHZUCHT PAUL EGGERS
D-2354 HOHENWESTEDT, FRG

BREEDER: CF. SUPPLIER

BODY LENGTH: 7.3 CM (RANGE: 6.9 - 7.9)

BODY WEIGHT: 3.4 G (RANGE: 2.5 - 5.0)

CORPULENCE FACTOR OF THE BATCH*: 0.9 (DETERMINED SEP. 5, 1989)

POSITIVE CONTROL OF ANIMALS CONDUCTED WITH CHLOROACETAMIDE
LC 50 AFTER 48 H: ABOUT 32 MG/L (DETERMINED AUG. 28, 1989)

THIS LETHAL CONCENTRATION CORRESPONDS TO THE NORMAL SENSITIVITY.

HOUSING:

THE FISH WERE KEPT IN A FLOW-THROUGH TANK IN TAP WATER CLEANED
BY ACTIVE CARBON AND AERATED WITH OIL-FREE AIR.

PHOTOPERIOD: 16 HOURS LIGHT AND 8 HOURS DARK

TOTAL HARDNESS : ABOUT 2.5 MMOL/L

ACID CAPACITY : ABOUT 5.5 MMOL/L

OXYGEN CONTENT : > 60% OF MAXIMUM SATURATION

PH : ABOUT 8.0

DURATION OF HOUSING AND ADAPTATION: ABOUT 4 MONTHS

WATER TEMPERATURE: 19 - 22 CENTIGRADE

MORTALITY DURING THE LAST 2 WEEKS OF HOUSING: ABOUT 1.1%

MORTALITY DURING THE ADAPTATION PERIOD: 0%

MEDICAL TREATMENT: TWICE WITH 0.05 MG/L MALACHITE GREEN CHLORIDE
ONCE WITH 10 MG/L TETRACYCLINE HYDROCHLORIDE

DIET: GROWING FEED F/B 50
SSNIFF SPEZIALDIAETEN GMBH
D-4770 SOEST, FRG
AD LIBITUM

* THE CORPULENCE FACTOR K IS CALCULATED FROM THE WEIGHT W (G) AND
THE LENGTH L (CM) MEASURED FROM THE TIP OF THE MOUTH TO THE DISTAL
END OF THE CAUDAL FIN ACCORDING TO THE FORMULA $K=100 \cdot W/L^{**3}$.

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TEST PROCEDURE:

THE STUDY WAS CARRIED OUT ACCORDING TO THE SPECIFIC STANDARD
OPERATING PROCEDURES (SOP) OF THE LABORATORY OF FISH TOXICOLOGY.

TEST WATER: RECONSTITUTED FRESHWATER ACCORDING TO
DIN 38 412, PART 11, OCTOBER 1982
PREPARATION FROM FULLY DEMINERALIZED TAP WATER
CONDUCTIVITY: MAX. 10 MICRO MHO

THE RESALTING IS CARRIED OUT BY ADDITION OF

294.0 MG/L $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$
123.3 MG/L $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$
63.0 MG/L NaHCO_3
5.5 MG/L KCL

CONTINUOUS AERATION WITH OIL-FREE AIR

TEST WATER READY FOR USE:

TOTAL HARDNESS : 2.5 MMOL/L
ACID CAPACITY : 0.8 MMOL/L
RATIO Ca/Mg IONS : 4 : 1
RATIO Na/K IONS : 10 : 1
PH : ABOUT 8.0

VOLUME OF WATER: 10 LITERS

NO. OF ANIMALS PER TEST CONCENTRATION: 10

LOADING (G FISH / L TEST WATER): 3.4

TEST VESSELS: ALL-GLASS AQUARIUM (30 CM X 22 CM X 24 CM)

TEST TEMPERATURE: 19 - 20 CENTIGRADE

ADAPTATION TO TEST WATER AND TEST TEMPERATURE: 3 DAYS

TEST PERIOD: SEP. 4 - SEP. 8, 1989

WITHDRAWAL OF FOOD: 1 DAY BEFORE AND DURING EXPOSURE

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EXAMINATIONS:

DETERMINATION OF MORTALITY AND SYMPTOMS;

DETERMINATION OR CALCULATION OF THE MEDIAN LETHAL CONCENTRATION (LC 50) AND, IF POSSIBLE, THE LC 5 AND THE LC 95 USING THE PROBIT ANALYSIS* AFTER HOURS (NOMINAL CONC.): 1, 4, 24, 48, 72, 96

REMARKS: NO UNDISSOLVED TEST SUBSTANCE WAS VISIBLE.

* FINNEY, D.J., PROBIT ANALYSIS, CAMBR. UNIV. PRESS, 3RD ED., 1971
CERTAIN ASPECTS OF THIS METHOD HAVE BEEN MODIFIED. THE EXACT
PROCEDURE CAN BE SEEN FROM THE STANDARD OPERATING PROCEDURES OF
THE LABORATORY.

RESULTS :

NOMINAL CONC. OF FISH (MG/L)	NUMBER OF FISH	DEAD FISH AFTER					
		1 H	4 H	24 H	48 H	72 H	96 H
0.100	10	0	0	0	0	0	0
0.215	10	0	0	0	0	0	0
0.464	10	0	0	0	0	0	0
1.000	10	0	0	0	0	0	0
2.150	10	0	0	0	0	0	0
4.640	10	0	0	0	0	0	0
10.000	10	0	0	10	10	10	10
0.000*	10	0	0	0	0	0	0

NOMINAL CONC. (MG/L)	SYMPTOMS					
	1 H	4 H	24 H	48 H	72 H	96 H
0.100						
0.215						
0.464						
1.000						
2.150						
4.640				A	A	
10.000		T				
0.000*						

EXPLANATION OF SYMPTOMS:

A=APATHY	B=ABDOMINAL DISTENSION
E=EXOPHTHALMOS	F=ESCAPE REFLEX
H=HYPERREFLEXIA	K=CONVULSIONS
L=GASPING	N=NARCOTIC-LIKE STATE
T=TUMBLING	U=RESTLESSNESS
V=DISCOLORATION	W=HEADSTAND
X=ACCELERATED RESPIRATION	Y=

* = SOLVENT CONTROL

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RESULTS :

NOMINAL CONC. (MG/L)	PH					
	1 H	4 H	24 H	48 H	72 H	96 H
0.100	7.2		7.3	7.3	7.3	7.3
0.215	7.2		7.3	7.3	7.3	7.3
0.464	7.2		7.3	7.4	7.3	7.3
1.000	7.3		7.3	7.4	7.3	7.3
2.150	7.3		7.3	7.4	7.3	7.3
4.640	7.2		7.3	7.4	7.3	7.4
10.000	7.3		7.4			
0.000*	7.3		7.4	7.4	7.4	7.4

NOMINAL CONC. (MG/L)	OXYGEN CONTENT (MG/L)					
	1 H	4 H	24 H	48 H	72 H	96 H
0.100	7.2		8.4	8.5	8.7	8.5
0.215	7.3		8.2	8.8	9.0	8.8
0.464	7.4		8.3	8.7	8.8	9.0
1.000	7.2		8.4	8.6	8.5	8.6
2.150	7.6		8.1	8.4	8.7	8.8
4.640	7.0		8.4	8.5	8.5	8.8
10.000	7.4		8.5			
0.000*	7.6		8.5	8.5	8.7	8.8

* = SOLVENT CONTROL

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RESULTS :

NOMINAL CONC. (MG/L)	TEMPERATURE (CENTIGRADE)					
	1 H	4 H	24 H	48 H	72 H	96 H
0.100	20		19	19	20	20
0.215	20		20	20	20	20
0.464	20		20	20	20	20
1.000	20		20	20	20	20
2.150	20		20	20	20	20
4.640	20		20	20	20	20
10.000	20		20			
0.000*	20		19	19	20	20

* = SOLVENT CONTROL

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STATISTICAL EVALUATION

NOMINAL CONCENTRATION

FISH TOXICITY AFTER	1 H	PAGE 11
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NOMINAL CONCENTRATIONS

FISH TOXICITY : AFTER 1H

NOMINAL CONC. (MG/L)	NUMBER OF FISH START	DEAD FISH AFTER 1H	MORT. (%)	CONC. USED
0.100	10	0	0.0	
0.215	10	0	0.0	
0.464	10	0	0.0	
1.000	10	0	0.0	
2.150	10	0	0.0	
4.640	10	0	0.0	
10.000	10	0	0.0	*

LC50 > 10.000 (1% SIGNIFICANCE LEVEL)

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NOMINAL CONCENTRATIONS

FISH TOXICITY : AFTER 4H

NOMINAL CONC. (MG/L)	NUMBER OF FISH START	DEAD FISH AFTER 4H	MORT. (%)	CONC. USED
0.100	10	0	0.0	
0.215	10	0	0.0	
0.464	10	0	0.0	
1.000	10	0	0.0	
2.150	10	0	0.0	
4.640	10	0	0.0	
10.000	10	0	0.0	*
LC50 >	10.000	(1% SIGNIFICANCE LEVEL)		

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NOMINAL CONCENTRATIONS

FISH TOXICITY : AFTER 24H

NOMINAL CONC. (MG/L)	NUMBER OF FISH START	DEAD FISH AFTER 24H	MORT. (%)	CONC. USED
0.100	10	0	0.0	
0.215	10	0	0.0	
0.464	10	0	0.0	
1.000	10	0	0.0	
2.150	10	0	0.0	
4.640	10	0	0.0	*
10.000	10	10	100.0	*
LC50 >	4.640	(1% SIGNIFICANCE LEVEL)		
LC50 <	10.000	(1% SIGNIFICANCE LEVEL)		

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NOMINAL CONCENTRATIONS

FISH TOXICITY : AFTER 48H

NOMINAL CONC. (MG/L)	NUMBER OF FISH START	DEAD FISH AFTER 48H	MORT. (%)	CONC. USED
0.100	10	0	0.0	
0.215	10	0	0.0	
0.464	10	0	0.0	
1.000	10	0	0.0	
2.150	10	0	0.0	
4.640	10	0	0.0	*
10.000	10	10	100.0	*
LC50 >	4.640	(1% SIGNIFICANCE LEVEL)		
LC50 <	10.000	(1% SIGNIFICANCE LEVEL)		

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NOMINAL CONCENTRATIONS

FISH TOXICITY : AFTER 72H

NOMINAL CONC. (MG/L)	NUMBER OF FISH START	DEAD FISH AFTER 72H	MORT. (%)	CONC. USED
0.100	10	0	0.0	
0.215	10	0	0.0	
0.464	10	0	0.0	
1.000	10	0	0.0	
2.150	10	0	0.0	
4.640	10	0	0.0	*
10.000	10	10	100.0	*
LC50 >	4.640	(1% SIGNIFICANCE LEVEL)		
LC50 <	10.000	(1% SIGNIFICANCE LEVEL)		

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NOMINAL CONCENTRATIONS

FISH TOXICITY : AFTER 96H

NOMINAL CONC. (MG/L)	NUMBER OF FISH START	DEAD FISH AFTER 96H	MORT. (%)	CONC. USED
0.100	10	0	0.0	
0.215	10	0	0.0	
0.464	10	0	0.0	
1.000	10	0	0.0	
2.150	10	0	0.0	
4.640	10	0	0.0	*
10.000	10	10	100.0	*
LC50 >	4.640	(1% SIGNIFICANCE LEVEL)		
LC50 <	10.000	(1% SIGNIFICANCE LEVEL)		