Exhibit 1

Effect of Polychlorinated Biphenyls (PCB's) on Survival and Reproduction of Daphnia, Gammarus, and Tanytarsus

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Abstract

Continuous-flow and static bioassays were conducted at 18 C, with survival and reproduction as measures of relative toxicity of eight PCB's, Aroclor 1221 (A-1221), A-1232, A-1242, A-1248, A-1254, A-1260, A-1262, and A-1268. Three PCB-mixture bioassays were also conducted. Aroclor 1248 was the most toxic to Daphnia magna of the eight Aroclors tested in static tests; the 3-wk LC50 was 25 µg/liter. Aroclor 1254 was the most toxic PCB to Daphnia under continuous-flow conditions with a 3-wk LC50 of 1.3 µg/liter. Ninety-six-hr LC50 values for A-1242, and A-1248 on Gammarus pseudolimnaeus in continuous-flow tests were 73 and 20 µg/liter. Survival after 60 days was 52% at 8.7 µg/liter 1242 and 53% at 5.1 µg/liter A-1248. Reproduction and survival of young were good at 2.8 µg/liter A-1242 and 2.2 µg/liter A-1248. The midge Tanytarsus dissimilis, in continuous-flow tests, did not emerge in abundance above 5.1 µg/liter A-1248 or 3.5 µg/liter A-1254. The 3-wk LC50 for Aroclor 1254 was 0.65 µg/liter for larvae and 0.45 µg/liter for pupae. Tissue residues in Gammarus pseudolimnaeus ranged from 4.0 µg/gram A-1254 in control animals to 552 µg/gram A-1248 in scuds held for 60 days in water containing 5.1 µg/liter A-1248.

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Introduction

Polychlorinated biphenyls (PCB's) are widespread in the environment, rivaling DDT in general occurrence (Risebrough and Bodine 1970). Their significance in the aquatic environment as toxicants is now being revealed. Duke, Lowe, and Wilson (1970) have documented the occurence of one of the many PCB mixtures (Aroclor 1254) in the water, sediment, and biota of Escambia Bay, Florida. Veith and Lee (1970) reviewed chlorinated biphenyl contamination in natural waters and stated that PCB's may be among the more widespread contaminants. A general review of the structural and physical properties, uses, analytical methods of analysis, concentrations found in nature, and toxicology of PCB's is given by Peakall and Lincer (1970). The acute toxicity of some of the many types of PCB's produced commercially (Monsanto Chemical Co.) has been demonstrated for a few species of fish and fish-food organisms (Stalling 1970; Zitco 1970; Nebeker, Puglisi, and DeFoe 1972; Duke, personal communication).

To assess the danger of the compounds to fish-food organisms, bioassays were designed and conducted with Daphnia magna as the initial test animal. All eight commercially available PCB's-Aroclor 1221 (A-1221) A-1232, A-1242, A-1248, A-1254, A-1260, A-1262, and A-1268—were tested. Seven continuous-flow bioassays were carried out with Aroclor 1248 and A-1254, twenty-four 3-wk static bioassays with all eight Aroclors, and three bioassays with PCB mixtures. Survival and reproductive success of Daphnia magna were used as measures of toxicity. The effects of A-1242 and A-1248 on the growth, survival, and reproduction of

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Gammarus pseudolimnaeus and the effects of A-1248 and A-1254 on the growth, survival, and adult emergence of Tanytarsus dissimilis were also determined. Tissue residues were also determined in Gammarus to measure uptake of PCB's.

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Materials and Methods

Physical Testing Methods

Daphnia static tests—Test containers were 250-ml glass beakers which were randomly distributed and covered with glass sheets to minimize evaporation. The experiments were conducted at 18 ± 1 C; the photoperiod was set for 16 hours light per day. Raw Lake Superior water was used for all testing.

Daphnia continuous-flow tests—*Daphnia*, *Gammarus*, and *Tanytarsus* were tested in a continuous-flow system which consisted of 24 stainless steel and glass 7.6 liter (2-gal) aquaria and which permitted the testing of five concentrations and a control in quadruplicate. A proportional diluter (Mount and Brungs 1967) provided the necessary continuous-flow conditions. A gas syringe filled with acetone and PCB was used with the diluter system to deliver the toxicant to the mixing chamber of the diluter. Additional agitation was maintained by circulating the mixing-chamber contents through a submersible pump. Mixing boxes with four-way splits to the test aquaria provided further mixing of the PCB solutions. All tanks were randomly distributed on the test tables. Raw Lake Superior water was used for all testing. The diluter delivered 150 ml to each test tank every 3 minutes. This flow rate ensured adequate dissolved oxygen levels, replenishment of fresh toxic solutions, and a complete exchange of water in the test tanks every 4-5 hours.

Test temperatures were maintained at 18 ± 1 C. Duro-test[2] (Optima FS) and wide spectrum Grow-lux fluorescent tubes provided light for all

[2]Mention of commercial products does not constitute endorsement by the U. S. Environmental Protection Agency.

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tests. The photoperiod of Evansville, Indiana, was maintained during testing, and adjustments in day length were made every 2 wks, as recommended by the Bioassay Methods Committee, National Water Quality Laboratory (Bioassay Committee MS 1971).

Biological Testing Methods

Daphnia static tests (single PCB)—Daphnia magna, obtained from the National Water Quality Laboratory cultures, were tested from young stages less than 24 hrs old through maturation, reproduction, and growth of their young (3 wks). Five animals were placed in a 250-ml beaker containing 200 ml of test solution; four duplicates gave 20 test animals at each concentration. The survival of the original animals was recorded each week, and the test solutions were renewed. The young surviving after 2 wks were counted and discarded. Young surviving after the third week were also counted, and the total number of young surviving was recorded. All tests were repeated three times.

The 3-wk LC50, that concentration at which 50% of the animals had died after 3 wks, was used as one measure of toxicity. Fifty percent and 16% reproductive impairment were used as measures of reproductive success (Biesinger and Christensen 1972). These values describe the survival of young as the percentage decrease from the highest number of young produced in either the control or one of the lower tested toxicant concentrations. The LC50, 50% and 16% reproductive impairment values, and 95% confidence limits for each were determined by the statistical method of Litchfield and Wilcoxon (1949). The terminology recommended by Sprague (1969) was used to present toxicity data.

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Food for the Daphnia was prepared from 0.5 g powdered dried wheat leaves and 10 g enriched trout food (fry granules) mixed in a blender with 300 ml of Lake Superior water (Biesinger and Christensen 1972).

Daphnia static mixture tests—Different percentages of a mixture of all eight PCB's were tested to determine the percentage survival in an aquatic system containing all the PCB's at low concentrations which barely permit reproduction. Testing methods were the same as those used in previous Daphnia static tests, and 50% survival and 50% and 16% reproductive impairment were also calculated as previously. The mixture consisted of the following concentrations (in µg/liter:) A-1221, 89; A-1232, 53; A-1242, 48; A-1248, 16; A-1254, 18; A-1260, 22; A-1262, 24; and A-1268, 162.

Daphnia continuous-flow tests—Five animals, less than 24 hrs old, were placed into 7.6 liter (2-gal) aquaria; four aquaria gave 20 test animals at each concentration. All surviving original Daphnia and their young were counted at the end of each 2- or 3-wk test. Analysis of data and physical-chemical conditions were the same as for the static tests; however, the animals were fed finely powdered fish food twice a day rather than the solution used in static tests. Mean measured PCB concentrations from the continuous-flow test tanks were used for all calculations.

Gammarus continuous-flow tests—Juvenile scuds, Gammarus pseudolimnaeus, from the Eau Claire River (Wisconsin) were held in the laboratory for at least 1 wk prior to testing. Twenty animals were placed in each tank; two (A-1242 test) and three (A-1248 test) replicate

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tanks for each PCB concentration gave 40 (A-1242) and 60 (A-1248) scuds in each concentration. They were fed a combination of well-soaked maple and aspen leaves; dry commercial fish food; and algae, which were permitted to grow on the tanks before introduction of the test animals. Tests were maintained for 2 mo; they were started with juvenile scuds and were continued until the animals matured, coupled, and reproduced (1 mo). Reproduction was allowed to continue for 1 mo before the test was ended. The adults were counted, and the young were counted and weighed.

Midge continuous-flow tests—The species used was Tanytarsus (Paratanytarsus) dissimilis; its original source is unknown. It was tested with Daphnia as they are compatible. The midges were reared in extra tanks in the room so that many egg-carrying females were flying in the room at the beginning of the test. These females deposited eggs in all clean test tanks when the test was started, prior to introduction of Daphnia, and within a few hours all tanks contained larvae. This continuing random ovipositing provided an abundant supply of young midges, which lived in cases on the bottom of the aquaria, independent of the Daphnia. The occurrence of mature larval midge cases and pupal cases, which the growing larvae constructed, was used as one measure of growth and survival. The additional occurrence of pupae and cast pupal skins at the water surface indicated the success of adult emergence.

All mature larval and pupal cases were counted at the end of 3 wks. Tanks were observed daily to determine when successful adult emergence occurred. The 3-wk LC50 was based on 50% reduction of test animals, with the number of control midges being considered as 100%. This

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procedure was useful under the particular conditions of these tests, but it is not recommended as a routine method.

Analytical Methods

Static Daphnia tests—Test solutions were prepared by weighing the Aroclor samples on an analytical balance and transferring them to volumetric flasks with acetone and Triton X-100, a surfactant (maintained at 0.03 x PCB level) added to help keep the PCB's solution. The concentrations of acetone and Triton X-100 used were tested previously and found to be nontoxic at the levels used during testing. The Triton-PCB mixtures were diluted to 500 ml with acetone and used as stock solutions. Portions of each stock solution were diluted with acetone to give five different working stock solutions for each Aroclor. One ml of each stock solution and 1 ml of food suspension were pipetted into a volumetric flask and diluted to 1,000 ml with Lake Superior water. Control solutions were prepared with 1 ml of acetone and 1 ml of food suspension in a liter of lake water to keep the acetone concentration constant (1 ml/liter) in all test solutions.

Continuous-flow tests—Methodology for preparation of stock solutions, diluter delivery systems, and analysis of PCB concentrations (gas chromatography) was identical to that used in earlier studies by Nebeker, Puglisi, and DeFoe (in press) with fathead minnows.

Chemical characteristics of the Lake Superior test water were monitored daily or weekly, and measurements were made with methods described by the American Public Health Association (1965). Dissolved oxygen in the tanks was measured by the azide modification of the Winkler method. Chemical characteristics were: pH, 7.5-8.0; acidity, 2.4-4.0 mg/liter; alkalinity, 40-43 mg/liter (as $CaCo_3$); calcium hardness, 34-36 mg/liter; and total hardness, 44-46 mg/liter.

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A standard one/two factor analysis-of-variance program was used to test for experimental differences.

Terminal residues of Aroclor 1242 and A-1248 in Gammarus were determined by the Fish-Pesticide Laboratory, Columbia, Missouri, using the methods of Stalling and Johnson (1971). Residues of PCB's in feed used in tests were also determined (Table 1).

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Results and Discussion

Daphnia Static Tests

Of the PCB's tested A-1248 was the most toxic to Daphnia; the 3-wk LC50 was 25 µg/liter. Fifty percent reproductive impairment occurred at 24 µg/liter, and 10% impairment was at 16 µg/liter. The toxicity of the other PCB's decreased progressively from A-1248 to A-1221 and from A-1248 to A-1268 (Table 2). Aroclors A-1254, A-1260, and A-1262 were nearly as toxic as A-1248, and A-1242 and A-1232 were about half as toxic as A-1248. Aroclor-1221 was much less toxic; the LC50 was 180 µg/liter. The least toxic was A-1268, with an LC50 of 253 µg/liter. In all tests reproduction, as judged by the number of young produced, was impaired by lower concentrations of Aroclor than was required to cause death. Values for 50% reproductive impairment of all PCB's tested were lower than the LC50 for survival. Excepting A-1221 and A-1268 the confidence limits indicate that the values are not significantly different, however.

No survival or reproduction occurred at 40% of the PCB mixture after 3 wk. At 20% of the mixture there was 17% adult survival and 13% young survival (Table 3). Fifty percent of the adults survived at 15% of the mixture. Survival of young was reduced to 50% at 14% of the mixture, and 16% reproductive impairment occurred at 11% of the mixture. Good survival, growth, and reproduction occurred at and below 5% of the mixture.

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Daphnia Continuous-Flow Tests

Aroclor-1254 was most toxic to Daphnia under continuous-flow test conditions. There was no significant difference between the 2-wk LC50 of 1.8 µg/liter and the 3-wk LC50 of 1.3 µg/liter (Table 2). Reproductive

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impairment occurred at or just below those concentrations that prevented survival of the adults. Aroclor-1248 killed 50% of the adult Daphnia at 2.6 µg/liter, and 50% of the young survived at 2.1 µg/liter. Sixteen percent reproductive impairment occurred at 1.0 µg/liter for A-1248 and for A-1254 at 0.48 (2 weeks) and 1.0 (3 weeks) µg/liter (Table 2). Almost no reproduction occurred at 7.5 µg/liter A-1248 (Table 4). No reproduction occurred at 3.8 µg/liter A-1254, and no adults survived 3.5 µg/liter. The number of young produced ranged from 0.7 young per initial adult in 7.5 µg/liter A-1248 to 76 young per surviving adult in 0.45 µg/liter A-1254 (Table 4). The Aroclors were much more toxic in continuous-flow conditions due to continual replacement of fresh PCB. Almost one-third of the PCB added to the test water was lost to the air, with some lost to bacteria, algae waste materials, test container surfaces, and test animal tissues. The difference between static- and continuous-flow results illustrates how critical test methodology can be for certain chemicals.

Gammarus Tests

Ninety-six-hr LC50 values for Gammarus juveniles were calculated for Aroclor-1242 and A-1248 (American Public Health Association 1965). Aroclor-1248 was twice as toxic as A-1242; the mean LC50 was 29 µg/liter for A-1248 (four tests, range 26-32 µg/liter) and 73 µg/liter for A-1242 (two tests, 72 and 74 µg/liter). Survival of the initial test animals after 2 mo was significantly less than survival after 96 hr (Tables 5 and 6). No live animals remained at 26 µg/liter A-1242 or 18 µg/liter A-1248. Fifty-two percent were alive at 8.7 µg/liter A-1242 (Table 5) and 53% were alive at 5.1 µg/liter A-1248 (Table 6). Good reproduction

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occurred at 2.8 µg/liter A-1242 and 2.2 µg/liter A-1248. Some reproduction occurred at 5.1 µg/liter A-1248, but it was only half that of the control and only one-fourth that of 2.2 µg/liter (Table 6). Terminal residues of A-1242 and A-1248 in Gammarus exposed for 60 days are given in Table 7, with control animal residue data summarized in Table 8.

Midge Tests

Adult midges emerged at concentrations up to 9 µg/liter A-1248. Larvae were present at 18 µg/liter, but adult emergence did not occur. Abundant emergence did not occur above 5.1 µg/liter. Arbclor-1254 was more toxic to midges as no emergence occurred above 3.5 µg/liter, and abundant emergence did not occur above 3 µg/liter, even though larvae were present (Table 9). Survival and growth of the midge when tested with A-1254 were excellent in control chambers, but were reduced by 50% at the lowest test concentration of 0.45 µg/liter (Table 9). At 1.2 µg/liter the numbers of larval cases were reduced to 35% of the control, and the numbers of pupal cases were reduced to 18% of the control. No larval cases were formed at 33 µg/liter, and no pupal cases were constructed at 9 µg/liter. The calculated 3 wk LC50 for A-1254 (50% reduction based on control as 100%) was 0.65 µg/liter for larvae and 0.45 µg/liter for pupae, indicating that a safe level of A-1254 for midge well-being is below 1 µg/liter.

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Table 1. Residues in feed used in tests

	Pesticide and PCB (µg/gram)				
	p,p'-DDE	p,p'-DDD	p,p'-DDT	Aroclor 1248	Aroclor 1254
Fish food Jan., 1971	0.22	0.02	0.10	0.3	0.8
Fish food Feb., 1971	0.20	0.02	0.10	0.5	1.5

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Table 2. Calculated 2- and 3-wk LC50 and 16% reproductive impairment values for Daphnia magna subjected to Aroclor 1248 and Aroclor 1254 in continuous-flow conditions and eight Aroclors in static conditions at 18 ± 1 C

Aroclor (PCB)	3-wk LC50 (µg/liter)	Confidence limits (0.05)	Reproductive impairment (µg/liter) 50%	16%
		Static test conditions		
A-1221	180	158.0-205.0	125	89
A-1232	72	62.6-82.8	66	53
A-1242	67	55.4-81.0	63	48
A-1248	25	21.4-29.2	24	16
A-1254	31	25.8-37.2	28	18
A-1260	36	27.7-46.8	33	22
A-1262	43	37.0-49.9	41	24
A-1268	253	222.0-288.0	206	162
		Continuous-flow test conditions		
A-1248	2.6 (2-wk LC50)		2.1	1.0
A-1254	1.8 (2-wk LC50)		1.1	0.48
A-1254	1.3 (3-wk LC50)		1.3	1.0

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Table 3. Survival and reproduction of Daphnia magna after a 3 week exposure to various percentages of a PCB mixture of 16% impairment concentrations at 18 ± 1 C[a]

Percentage of mixture[b]	Initial number of animals	Adults alive at end of test			Mean survival of adults (%)	Total young produced			Mean young produced[c] (%)
		Test 1	Test 2	Test 3		Test 1	Test 2	Test 3	
40	20	0	0	0	0[d]	0	0	0	0[d]
20	20	2	8	0	17[d]	80	152	68	13[d]
10	20	16	20	20	93	554	792	631	87
5	20	18	20	20	97	690	795	804	100
1	20	14	19	19	87	425	670	668	77
0.5	20	15	20	20	92	566	871	701	94
0.0	20	17	18	20	92	688	757	836	99

[a]Concentrations of each Aroclor that permitted reproduction, but reduced it by 16%.

[b]Mixture consisted of the following concentrations (in µg/liter): A-1221, 89; A-1232, 53; A-1242, 48; A-1248, 16; A-1254, 18; A-1260, 22; A-1262, 24; and A-1268, 162.

[c]Percentage of the highest young produced in one of the lower tested toxicant levels (5%).

[d]Significantly different from the controls at the 0.05 level.

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Table 4. Survival and reproduction of Daphnia magna after 2 weeks' continuous-flow exposure to Aroclor 1248 and Aroclor 1254, and 3 weeks' exposure to Aroclor 1254

Mean measured concentration (µg/liter)	Initial number of animals	Total number of adults alive at end of test	Mean survival of adults (%)	Total young produced	Young produced[a] (%)	Young per initial adult	Young per surviving adult
1248 (2 wk)							
7.5	60	3	5	43	2.6	0.7	14
2.5	60	39	65	699	43	12	18
0.86	60	55	92	1441	88	24	26
0.26	60	52	87	1641	100	27	31
0.1	50	37	74	627	46	13	17
0.0	50	30	60	786	56	16	26
1254 (2 wk)							
9.0	20	0	0	0	0	0	0
3.8	20	0	0	0	0	0	0
1.7	20	12	60	239	36	12	20
0.92	15	14	93	269	53	18	19
0.37	15	12	80	504	100	34	42
0.0	15	8	53	290	58	19	36
1254 (3 wk)							
33.0	40	0	0	0	0	0	0
9.0	40	0	0	0	0	0	0
3.5	40	0	0	0	0	0	0
1.2	40	28	70	1583	76	39	56
0.45	35	24	69	1816	100	52	76
0.0	30	24	80	1154	74	38	48

[a]Percent decrease from the highest number of young produced in either the controls or one of the lower tested toxicant levels.

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Table 5. Survival and reproduction of Gammarus pseudolimnaeus after 2 months' exposure to Aroclor 1242 in continuous-flow systems at 18 C

Mean measured concentration (µg/liter)	Initial number of animals	Total number of adults alive at end of test	Mean survival of adults (%)	Total young produced	Young per surviving adult
234	40	0	0	0	0
81	40	0	0	0	0
26	40	0	0	0	0
8.7	40	21	52	0	0
2.8	40	31	77	131	4.2
0.0	40	19	48	130	6.8

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Table 6. Survival and reproduction of Gammarus pseudolimnaeus after 2 month's exposure to Aroclor 1248

Mean measured concentration (µg/liter)	Initial number of animals	Total number of adults alive at end of test	Mean survival of adults (%)	Total young produced	Young per surviving adult	Total weight of young (mg)	Mean weight of each young scud (mg)
18	45	0	0	0	0	0	0
5.1	45	24	53	169	7.0	90	0.53
2.2	45	33	73	729	22.1	287	0.39
0.54	45	32	71	627	19.6	336	0.54
0.18	45	33	73	357	10.8	154	0.43
0.0	45	29	64	330	11.4	200	0.61

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Table 7. Terminal residues of Aroclor 1242 and Aroclor 1248 in *Gammarus* exposed for 60 days

Mean measured PCB concentration (µg/liter)	Residues (µg/gram) A	B	Mean	Concentration factor		Nominal Column
A-1242						
2.8	71(14)[a]	80(17)	76	22,000	27,000	3.5
8.7	246(9)	387(10)	316	29,000	36,000	11.0
26	409(1)	—	409	14,000	16,000	33.0
A-1248						
0.18	9(33)	—	9	24,000	50,000	0.37
0.54	15(32)	—	15	14,000	28,000	1.10
2.2	127(33)	—	127	38,000	58,000	3.3
5.1	552(24)	—	552	55,000	108,000	10.0

[a]Mean of (N) individual samples.

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Table 8. Terminal residues in control Gammarus from Aroclor 1242 study

Gammarus	p,p'DDE (µg/gram)	Aroclor 1248 (µg/gram)	Aroclor 1254 (µg/gram)
A Controls	0.12(7)[a]	8.0	4.0
B Controls	0.06(12)	6.0	7.0

[a]Mean of (N) individual samples.

Table 9. Effect of Aroclor 1254 on the growth and survival of the midge *Tanytarsus dissimilis*

Mean measured concentration (µg/liter)	Test	Number of mature larval cases: Each test	Mean	Percentage of control	Number of pupal cases: Each test	Mean	Percentage of control
33	A	0			0		
	B	0	0	0	0	0	0
	C	0			0		
	D	0			0		
9.0	A	0			0		
	B	0	0.25	0.2	0	0	0
	C	0			0		
	D	1			0		
3.5	A	3			1		
	B	7	8	7.4	0	2	6.8
	C	14			5		
	D	-			-		
1.2	A	43			7		
	B	68	37	35	5	5.2	18
	C	4			2		
	D	35			7		
0.45	A	70			4		
	B	25	56	52	27	16	55
	C	82			16		
	D	48			16		
0.0	A	94			32		
	B	108	107	100	33	29	100
	C	119			22		
	D	-			-		

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