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Organic Contaminants and Trace Metals in the Tissues of Green Turtles (*Chelonia mydas*) Afflicted with Fibropapillomas in the Hawaiian Islands

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Environmental contaminants have been listed as a possible cause of green turtle fibropapillomas (GTFP). Brain, fat, liver, and kidney tissues from 10 juvenile green turtles (*Chelonia mydas*) afflicted with GTFP, were tested to determine exposure to selected environmental pollutants and any possible relation to GTFP. One juvenile green turtle free of the disease, one pelagic green turtle, and one pelagic loggerhead turtle (*Caretta caretta*) served as controls. Egg shells and tissues from three green turtle hatchlings were also tested. The tissues and shells analysed in this study indicated that none contained any of the listed organochlorine, polychlorinated biphenyl, organophosphate, or carbamate insecticides in concentrations above the stated method of detection limits. Most of the concentrations of selenium and heavy metals were also considered to be below levels reported normal in other animal species. No correlation was found between the contaminants tested and GTFP because of the low levels detected. Trace metals and other pollutants tested in this study play a minor role in the aetiology of GTFP in a discrete green turtle population at Kaneohe Bay, Island of Oahu, Hawaii.

Green turtle fibropapillomatosis (GTFP) is a condition affecting several green turtle (*Chelonia mydas*) populations in epidemic proportions throughout the world. Although a virus has been suggested as the causative agent, the primary aetiology is unknown. Chemical pollutants impairing the immune system and stress have been listed among many other possible aetiologic agents (Aguirre, 1991, 1993; Balazs & Pooley, 1991).

Pollutant-mediated stress causing morphophysiological changes such as cellular proliferation (skin tumours) is well documented in aquatic organisms (Giam & Ray, 1987). This chronic stress results in a reduction of energy available for basic physiologic processes as well as causing alterations in the cellular immune response increasing susceptibility to infectious agents. Papillomas and other tumours in fish have been used as an index to monitor chemical carcinogens in the marine environment: relatively few studies, however, correlate the exposure of sea turtles and their eggs to organic pollutants (Hutchinson & Simmonds, 1991).

The purpose of this study was to determine the involvement of selected environmental pollutants on the aetiology of GTFP. Toxicological information was compared to determine baseline data for the accumulation of organochlorines (OCs), polychlorinated biphenyls (PCBs), organophosphates (OPs), n-methyl carbamate compounds, selenium (Se), and heavy metals in selected tissues collected from a discrete population of juvenile green turtles in Kaneohe Bay, Oahu, and elsewhere in the Hawaiian Islands.

Materials and Methods

Field sampling

Tissue specimens for this study were obtained from different sources. Four turtle carcasses were recovered through the National Marine Fisheries Service (NMFS) Hawaiian sea turtle stranding and salvage programme. Two of those turtles were found in a bull pen net at Palaau, Island of Molokai, one was found at Kaneohe Bay, Island of Oahu, and the fourth one was found at Waialae Beach Park, Island of Oahu. Six juvenile green turtles, severely affected by GTFP and in poor

condition, were caught by hand while snorkelling at Ahu-O-Laka (n=4) and Mark Reef (n=2), Kaneohe Bay, by NMFS personnel and transported to the Honolulu Laboratory for euthanasia and necropsy. Subcutaneous fat, kidney, liver, and brain tissues were collected for toxicological analysis from each of these turtles. One clinically normal green turtle held in captivity for 5 years at the NMFS research facilities because of traumatic amputation of front flippers, served as a control. Tissue samples from one pelagic green turtle and one pelagic loggerhead turtle (*Caretta caretta*) free of GTFP were also tested as controls (Table 1). These two turtles had been salvaged as the result of incidental mortality in the foreign driftnet fishery. In addition, we tested tissues and shells from three green turtle hatchlings collected at French Frigate Shoals, Hawaii, from the nest of a turtle known to be afflicted by GTFP.

The seven live turtles were euthanized with a lethal intraperitoneal injection of T-61 Euthanasia Solution (American Hoechst Corp., Somerville, New Jersey) and thorough necropsies were performed according to a protocol previously described (Wolke & George, 1981). A sample of 50–100 g of liver and adipose tissue, and the whole brain with cervical spinal cord specimens were wrapped in acetone-rinsed (and dried) aluminium foil, then labelled and placed in double zip-lock bags for organic contaminant analysis. Liver and kidney specimens (50 g) were placed in double zip-lock bags for heavy metal analysis. All specimens were frozen immediately on dry ice and shipped overnight to the laboratory for analysis.

Cholinesterase activity

The quantitative determination of acetylcholinesterase (ChE) activity in brain specimens was performed with a plate reader. The principle of this method is the measurement of the rate production of thiocholine as described by Ellman *et al.* (1961). The original Ellman assay was adopted for a 96-well kinetic microplate reader (Richardson *et al.*, 1993). The activity of ChE was expressed in μm of acetylthiocholine hydrolysed per gram of brain sample per minute (μm g^{-1} min^{-1}). Specimens were homogenized, diluted with pH 8.0, phosphate buffer and pipetted into wells of the microplate. DTNB (Ellman reagent) and ATCI (substrate solution) were then added and the A^{405} was measured every 8 s for 5 min by a microplate reader. The method detection limit (MDL) was 0.1 μm g^{-1} min^{-1}.

Multiresidue pesticide screen

Tissue specimens were prepared for a multiresidue screen of selected OCs, PCBs, OPs, and carbamates using a solvent extraction followed by gel permeation chromatography (GPC). Briefly, 10 g aliquots of selected tissues were homogenized in solvent. Subsamples (20 ml) were evaporated to dryness at 40°C with a stream of N_2. Then, 10 ml of hexane:ethyl acetate 7:3 (u:v) were added, filtered through a 0.45 μ filter and loaded onto GPC (Holstege *et al.*, 1991).

For OP analysis, 2 μl of extract and standard were injected on a HP5890 Gas Chromatograph/FPD(P) and a 30 m×0.53 mm×1 μm DB-17 column. For OC analysis, 2 ml extract and the standard were injected on a PE Sigma 2000 Gas Chromatograph/ECD, column 30 m×0.53 mm×0.83 μm DB-608. For carbamates, 10 μl of extract and standard were injected on a HP1090 Liquid Chromatograph with Hitachi F-1050 fluorescent detector and dual post column hydrolysis/reaction. Gas chromatography/mass spectrometry was required to completely exclude the presence of p,p'DDD, gamma chlordane, lindane, and heptachlor in several samples, since initial screening for those compounds was inconclusive. The MDL was 0.1 ppm wet wt for carbamate residues and 1 ppm for PCBs. Method detection limits for OCs and OPs are stated in Table 2 (Holstege *et al.*, 1991; Holstege, 1992).

TABLE 1

Size, weight, sex, and brain cholinesterase activity levels (ChE) for 12 green turtles (*Chelonia mydas*) sampled from the Hawaiian Islands.

Turtle no.	Location	SCL* (cm)	WGT† (kg)	Sex	ChE‡ (μm g^{-1} min^{-1})
035	Palaau, Molokai	50.8	15.5	F	3.6
036	Palaau, Molokai	58.6	22.7	F	3.5
037	Mark Reef, Kaneohe	46.0	10.0	M	5.4
043	Control, captive	52.3	16.4	F	6.2
044	Waialae Beach Park	49.5	16.4	F	4.5
045	Kaneohe Bay, Oahu	69.0	42.3	F	3.2
046	Ahu-O-Laka, Kaneohe	56.1	18.2	F	7.9
047	Ahu-O-Laka, Kaneohe	52.3	16.8	F	3.7
048	Mark Reef, Kaneohe	71.3	43.6	M	5.4
049	Ahu-O-Laka, Kaneohe	52.9	20.0	F	5.9
050	Ahu-O-Laka, Kaneohe	66.9	37.5	M	6.8
051	Pelagic green	28.7	3.2	M	11.7

*SCL (cm)—Straight Carapace length (centimetres).
†WGT (kg)—Weight (kilograms).
‡The method detection limit was 0.1 μm g^{-1} min^{-1}.

Total selenium

Total Se was analysed by inductively-coupled plasma (ICP) atomic emission using hydride vapour generation. The instrument MDL was determined to be 0.007 ppm wet wt (Tracy & Möller, 1990).

Extended heavy metal screen

The preparation of kidney and liver specimens for the analysis of heavy metals was a simple nitric acid digestion of a 1 g sample diluted to 10 ml of distilled deionized water. Samples were analysed in an ICP spectrophotometer screening for 16 heavy metals (Table 3) (Tracy & Melton, 1988).

Results

Cholinesterase activity

Cholinesterase levels were determined in 12 brain samples from green turtles. The brain samples contained the listed ChE activities within expected ranges for most animals. Turtles captured at Kaneohe Bay had a mean (±SE) ChE activity of 5.85 (±1.3) μm g^{-1} min^{-1}. Turtles recovered through the stranding programme had mean (±SE) ChE activity of 3.7

TABLE 2

Organochlorines, polychlorinated biphenyls (PCBs), carbamates, and organophosphates with method of detection limits (MDL) in parts per million (ppm) wet wt; analysed in selected green turtle (*Chelonia mydas*) tissues from the Hawaiian Islands. None of the tissues exceeded these MDL levels.

Organochlorines	MDL (ppm)	Organophosphates	MDL (ppm)
Aldrin	0.02	Acephate	0.025
BHC	0.02	Azinphos-methyl	0.025
Chlordane	0.25	Carbophenothion	0.025
p,p'-DDD	0.1	Chlorfenvinphos	0.02
o,p'-DDD	0.1	Chlorpyrifos	0.02
p,p'-DDE	0.1	Coumaphos	0.025
o,p'-DDE	0.1	Crotoxyphos	0.025
p,p'-DDT	0.1	Cruformate	0.025
o,p'-DDT	0.1	DDVP	0.025
Dicofol	0.1	DEF	0.01
Dieldrin	0.02	Demeton	0.03
Endosulfan I	0.02	Diazinon	0.01
Endosulfan II	0.02	Dicrotophos	0.025
Endrin	0.02	Dimethoate	0.02
Gamma chlordane	0.02	Dioxathion	0.05
HCB	0.02	Disulfoton	0.02
Heptachlor	0.05	EPN	0.025
Heptachlor epox	0.02	Ethion	0.01
Lindane	0.05	Ethoprop	0.025
Methoxychlor	0.04	Fenamiphos	0.025
Mirex	0.04	Fensulfothion	0.025
Oxychlordane	0.05	Fenthion	0.025
Toxaphene	0.5	Fonophos	0.025
		Isofenphos	0.025
PCBs		Malathion	0.01
Aroclor 1016	1.0	Merphos	0.02
Aroclor 1221	1.0	Methamidaphos	0.025
Aroclor 1232	1.0	Methidathion	0.02
Aroclor 1242	1.0	Methyl parathion	0.01
Aroclor 1248	1.0	Mevinphos	0.01
Aroclor 1254	1.0	Monocrotophos	0.025
Aroclor 1260	1.0	Naled	0.04
Aroclor 1262	1.0	Parathion	0.01
		Phorate	0.01
Methyl carbamates		Phosalone	0.025
Aldicarb	0.1	Phosmet	0.05
Bendiocarb	0.1	Phosphamidon	0.05
Carbaryl	0.1	Profenophos	0.025
Carbofuran	0.1	Propetamphos	0.025
Methiocarb	0.1	Ronnel	0.025
Methomyl	0.1	Terbufos	0.025
Mexacarbate	0.1	Tetrachlorvinphos	0.025
Oxamyl	0.1	Triazophos	0.025
Propoxur	0.1		
Aldicarb sulfon	0.1		
3-Hydroxy carbo	0.1		

(± 0.5) $\mu m\ g^{-1}\ min^{-1}$, and the pelagic green with a ChE activity of 11.7 $\mu m\ g^{-1}\ min^{-1}$. The control turtle had a ChE level of 6.2 $\mu m\ g^{-1}\ min^{-1}$ (Table 1). A one-way analysis of variance demonstrated significant differences among the stranded group and the other turtle groups ($p < 0.05$). The pelagic green had higher ChE activity than the other turtles. Also, the group of turtles trapped at Kaneohe Bay had higher levels than the stranded turtles. Similar ChE activity levels were recorded between the Kaneohe Bay turtle group and the control turtle.

Multiresidue pesticide screen

Extended multiresidue screenings were performed for 23 OC, 8 PCB, 43 OP, and 11 carbamate insecticides (Table 2). Liver, kidney, and adipose tissues from the green turtle specimens contained none of the organophosphorus, organochlorine, and carbamate insecticides or PCBs in concentrations greater than the stated MDL: that is, the lowest concentration detectable by the test method sensitivity implemented in this study. Additional testing was required to exclude the presence of heptachlor (6 samples), lindane (6 samples), and p,p'DDD (2 samples).

The egg shells and hatchling tissues contained none of the listed OC insecticides or PCBs in concentrations above the stated MDL (Table 2). Additional testing was required to rule out the presence of gamma chlordane in the hatchling tissues.

Selenium and heavy metals

The concentrations of selenium and 16 heavy metals detected on selected samples of sea turtles are summarized in Table 3. Most tissue samples contained none of the listed metals or selenium in toxic concentrations for most animals. The liver samples collected from the pelagic green and loggerhead turtles, however, contained relatively high selenium levels. 2.53 ppm and 3.39 ppm wet wt respectively (normal concentrations for most terrestrial animal livers are < 1.5 ppm). Four

TABLE 3

Selenium and heavy metal concentrations in parts per million (ppm) wet wt detected in liver (L) and kidney (K) tissues of green turtles (*Chelonia mydas*), Hawaiian Islands.

Heavy metals (MDL)*	Turtle identification numbers																																			
	035		036		037		043		044		045		046		047		048		049		050		051		052	060	061	
	L	K	L	K	L	K	L	K	L	K	L	K	L	K	L	K	L	K	L	K	L	K	L	K	L	GTH†	TES‡	
Selenium 0.007	0.576	0.159	0.385	0.194	1.36	0.957	0.999	0.700	0.136	0.223	0.335	0.186	0.844	0.445	0.899	0.390	0.316	0.231	0.657	0.283	0.457	0.208	2.53	1.58	3.39	NT§	NT	
Thallium 0.7	(—)‖	—	—	—	—	—	—	—	—	—	—	—	1.0	—	—	—	1.0	—	0.8	—	0.9	—	—	—	1.1	—	—	
Arsenic 0.6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6.4	6.8	0.9	—	—	
Molybdenum 0.1	0.3	—	0.1	0.2	0.3	0.1	0.1	0.1	—	—	—	0.1	0.4	0.2	0.2	0.2	0.2	0.3	0.6	0.3	0.2	0.2	—	0.2	0.2	—	—	
Zinc 0.03	26.5	12.7	39.5	18.4	45.8	38.1	37.8	31.7	19.7	17.3	19.6	12.5	40.6	26.3	33.6	21.6	34.2	27.1	41.9	22.7	25.1	20.0	18.1	19.1	15.1	12.1	6.25	
Cadmium 0.07	5.44	4.77	4.51	9.84	25.6	70.2	3.10	22.0	0.39	8.85	1.80	9.96	26.0	47.4	11.2	33.9	13.6	47.7	13.8	36.7	5.04	15.9	1.12	4.72	0.96	—	0.2	
Nickel 0.4	—	—	—	0.8	—	1.0	—	—	—	—	—	0.7	—	0.5	—	0.8	—	—	—	—	—	0.9	—	—	—	—	—	
Manganese 0.03	2.79	0.48	1.65	1.23	2.04	1.24	2.13	0.82	0.15	0.59	0.78	0.66	1.32	1.12	1.56	1.09	1.76	1.39	1.24	1.12	1.39	1.21	2.34	0.56	1.59	1.12	0.31	
Iron 0.2	1730.0	38.8	1620.0	126.0	2260.0	12.7	765.0	16.3	155.0	17.90	1330.0	23.0	2450.0	9.9	446.0	8.8	1250.0	10.5	1740.0	13.0	1290.0	14.9	101.0	66.5	92.8	128.0	14.1	
Chromium 0.2	—	—	—	—	0.2	—	0.5	0.4	—	—	—	—	0.2	—	—	—	—	—	—	—	0.2	—	—	—	—	—	0.4	
Aluminum 1.0	3.0	2.0	5.0	2.0	1.0	1.0	1.0	1.0	—	—	4.0	1.0	3.0	2.0	1.0	1.0	2.0	1.0	1.0	—	2.0	1.0	—	1.0	—	—	3.0	
Vanadium 0.2	0.2	—	0.2	—	0.7	0.7	0.3	—	—	—	0.9	—	1.2	—	0.4	—	0.3	—	1.5	0.3	0.9	—	0.8	2.5	—	—	—	
Copper 0.2	91.4	1.5	13.4	1.1	17.3	3.3	116.0	10.5	1.1	1.5	33.9	6.9	106.0	4.4	86.7	2.3	189.0	3.4	149.0	2.2	35.6	1.8	20.2	4.7	2.8	2.2	14.3	
Barium 0.07	0.58	0.82	0.61	1.58	0.75	1.01	0.70	0.88	0.58	0.76	0.74	1.0	0.82	0.90	0.69	0.98	0.63	0.86	0.78	0.94	0.66	0.91	0.63	0.98	0.83	0.35	1.0	

*MDL = method detection limits; †GTH = green turtle hatchlings; ‡TES = turtle egg shells; §= Not tested; ‖ = concentrations below MDL.

green turtles captured at Kaneohe Bay had evidence of hepatic trace levels of thallium (Tl) (0.8–1.0 ppm). All liver and kidney tissues, hatchlings, and egg shells from the turtles analysed in this study contained no beryllium, lead, or mercury in levels above the stated MDLs.

Discussion

Cholinesterase activity

Measuring the inhibition of ChE activity in brain tissue is a sensitive indicator of acute exposure to organophosphorus or carbamate insecticides, with a reduction of 20% normal activity suggesting exposure. Findings are difficult to interpret, however, because what is 'normal' ChE value for a given species must be determined before levels can be adequately assessed (Fairbrother & Bennett, 1988). At Johnston Atoll, south of the Hawaiian Islands, erythrocyte ChE was measured for nine green turtles by identifying changes in cellular pH (Balazs, 1985). That data set, however, is not comparable with the activities obtained in this study since different techniques and tissues were utilized. Comparative studies of fish, birds, and mammals on levels of ChE inhibition have shown remarkable differences in sensitivity to different OP and carbamate compounds (Hall, 1980). For example, amphibians apparently are less sensitive to OPs than other animal groups. More studies in reptilian sensitivity are required to determine the validity of the ChE inhibition test in sea turtles.

During this study, the lower values identified in the stranded turtles when compared to other turtles were considered normal since brain ChE activity is severely depressed by the time of death (Fairbrother & Bennett, 1988). Further studies are required to determine the significance of higher levels of ChE activity shown in the pelagic green when compared to other turtle groups.

Multiresidue pesticide screen

No OC, PCB, OP, or carbamate residues were detected in any of the green turtle tissues analysed. Based on the results obtained, juvenile green turtles in this study were not exposed to any of these pollutants at toxic levels at the time of sample collection.

Several investigators have measured organochlorine and PCB levels of green turtles and their eggs (Thompson *et al.*, 1974; Clark & Krynitsky, 1980, 1985; Rybitski, 1993). Trace levels of p,p'DDE and PCBs were detected in 10 green turtle egg yolks from Ascension Island, South Atlantic Ocean (Thompson *et al.*, 1974). DDE and DDT residues averaging 0.025 ppm wet wt were also found in nine clutches (170 eggs) of green turtles nesting on Merrit Island, Florida (Clark & Krynitsky, 1980). Post-yearling green turtles collected in Florida presented DDE levels of < 10 ppb and PCB levels of 43–80 ppb wet wt (McKim & Johnson, 1983). More recently, Rybitski (1993) determined PCB concentrations from minimum quantification limits to 58.2 ppb in subcutaneous fat and up to 17.1 ppb in livers of Hawaiian green turtles. DDE levels

in adipose tissue ranged from minimum quantification limits to 22.5 ppb and up to 6.31 ppb in liver.

Organochlorine and PCB compounds elicit many biological effects including birth defects, tumours, a wasting syndrome, and death. These compounds are know to bioaccumulate and biomagnify within the food chain. According to the studies mentioned above and confirmed by this study, OC and PCB residues exist at extremely low concentrations in this turtle species. In addition to species, sex, age, nutritional status, and exposure, the dietary habits of the species may explain these findings. Hawaiian green turtles feed primarily on marine algae and a sea grass making them less susceptible to bioconcentration of these pesticides.

To the best of our knowledge, no studies are available in sea turtles related to OP and carbamate toxicity. Many of these compounds are complex mixtures with components that are metabolized selectively by certain species and their quantification is difficult and has not been standardized. In addition to inhibition of ChE activity and delayed neurotoxicity, these compounds have other sublethal effects in wildlife including impaired reproduction in birds and reduced tolerance to cold stress (Smith, 1987). These compounds have a relatively low environmental persistence and their monitoring is very difficult. Brain ChE activity values, detection of residues in the gastrointestinal tract, and die-offs characterized by acute poisoning in a defined geographic area can confirm exposure to these pesticides.

Selenium

Selenium is nutritionally important as an essential trace element of many animals, but is toxic at slightly higher concentrations. Total background Se levels in sea turtles are unknown and most concentrations in this study were considered to be within levels reported as 'normal' for other animal species. Accumulation of Se in marine animals is highly variable, ranging from 0.05 ppm in crustaceans to 30 ppm wet wt in some bird and fish species. Levels in kidneys collected from marine birds have been recorded at 1.2–10.2 ppm wet wt. Levels of this magnitude are sufficient to impair reproduction in shorebirds (Eisler, 1985).

Marine mammal tissues contain extremely high Se concentrations. For example, hepatic levels in seals range from 6.1 ppm in pups to 170 ppm wet wt in adults. High hepatic concentrations of maternal California sea lions (*Zalophus californianus*) were not reflected in livers of pups (Eisler, 1985). This trend was not observed in the green turtles samples. The pelagic specimens had significantly ($p < 0.05$) higher Se levels than the turtles recruited at nearshore environments. Further research about Se in sea turtles and marine ecosystems is necessary.

Heavy metals

Sea turtles and their eggs have been analysed for traces of heavy metals (Hillestad *et al.*, 1974; Stoneburner *et al.*, 1980; Witkowski & Frazier, 1982; Balazs & Forsyth, 1986; Davenport & Wrench, 1990; Hutchinson & Simmonds, 1991). Concentrations of 13 heavy metals in eggs of loggerhead turtles provided useful information on feeding ecology of this species (Stoneburner *et al.*, 1980). In most cases, however, it was difficult to interpret the significance of these trace metals due to the lack of baseline data. The effects of these elements in sea turtles are unknown.

Thallium levels of Kaneohe Bay turtles were relatively high when compared to other animal groups. These levels may be nontoxic to sea turtles, and reflect exposure from their food source or the aquatic environment. Most cases reporting toxicity in wildlife and humans occur as acute poisonings. Chronic cases have not been well documented with the most consistently reported effect in terrestrial mammals being alopecia. The available data indicate that Tl acute toxicity to saltwater aquatic life occurs at concentrations as low as 2.13 ppm. No information was available concerning chronic toxicity of Tl to sensitive marine life (Anon., 1980).

Further research on other contaminants such as airplane pollutants, oil, and dioxin is recommended. Reported sublethal effects of oil ingestion include metabolic production of potential carcinogenic compounds, immunosuppression, diminished salt gland function, and hormonal and behavioural abnormalities (Hall *et al.*, 1983; Lutz *et al.*, 1986). In the absence of background information in the literature related to environmental pollutants in terrestrial or aquatic reptiles, data from other species indicated that the presence of these and other compounds represent an increased risk for sea turtles.

Conclusions

Reproductive failure, hormonal imbalance, low population recruitment, and suppression of the immune system are known results of exposure to persistent environmental pollutants. The possibility that these compounds may have deleterious effects on disease including GTFP, in green turtles is a consideration. Research in other species with fibropapillomas has suggested that chemical pollution may activate latent viruses or indirectly increase their virulence (Gamache & Horrocks, 1992). An immune-mediated component to the disease has been suggested (Balazs & Pooley, 1991; Hutchinson & Simmonds, 1991) implicating many pollutants as possible immunosuppressants. Based on this study, however, environmental contaminants studied herein do not appear to play a role in the aetiology of GTFP in this discrete green turtle population at Kaneohe Bay, Island of Oahu, Hawaii. Further research on infectious and parasitic agents is recommended.

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