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CHLORDANE RESIDUES IN GREAT LAKES LAKE TROUT: ACUTE TOXICITY AND INTERACTION AT THE GABA RECEPTOR OF RAT AND LAKE TROUT BRAIN

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Abstract-The assemblage of chlordane-related compounds present in Great Lakes lake trout is substantially different from the technical grade mixture. To investigate the toxicological consequences of this alteration, we isolated a mixture of chlordane related compounds from tissues of lake trout (Salvelinus namaycush) sampled from the southern end of Lake Michigan and from Siskiwit Lake on Isle Royale in Lake Superior. The toxicity of the residue was evaluated using an acute bioassay and a neuroreceptor binding affinity assay. We compared the toxicity of the isolated residue with the technical mixture and various combinations of pure components and found that residues were three to five times more toxic than the technical mixture. Our tests suggest that the increased toxicity is due to the presence of the stable metabolites, heptachlor epoxide and oxychlordane, and show a close correlation between variation in toxicity and the relative abundance of heptachlor epoxide. The substantial increase in the toxicity of the chlordane residue over the technical mixture suggests that caution should be excercised when making human or environmental health judgements regarding chlordane residues.

INTRODUCTION

Chlordane, a chlorinated hydrocarbon insecticide, was introduced in the late 1940's and was popular for control of a wide variety of pests (1). By 1974, commercial and home use comprised slightly over 60% of the total (2), while more recently it was used only for subterranean termite control. Because of its widespread application and persistence, chlordane residues can be found in

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freshwater fish from most of the rivers in the U.S. (3). In addition, chlordane residues have been detected in fish from the baltic (4), the canadian east coast (5), the arctic (7) and the antarctic (6), making it a prominent member of the global chlorinated hydrocarbon pollutants.

As with other complex mixtures, it is difficult to assess the possible impacts of chlordane residues. Technical chlordane is a mixture of approximately 50 components (8, 9), with cis- and trans-chlordane, the two most abundant and toxic components, comprising approximately 43% of the total (10). Other dominant components are chlordene isomers (ca. 25%), cis- and trans-nonachlors (ca. 10%) and heptachlor (ca. 7%). In addition, chemical residues of chlordane are composed of entirely different proportions of the original components, and often contain the metabolites oxychlordane and heptachlor epoxide (11). Heptachlor epoxide and oxychlordane are both more toxic than their parent compounds (12). Since much of the data regarding the toxicity and carcinogenicity of chlordane was established using the technical mixture or pure cis- and trans-chlordane (2), there is a need to conduct studies on the toxicological properties of chlordane residues. Testing of these residues, however, represents a significant challenge, since they are usually present with a variety of other toxic materials. In this study, we have purified and tested the acute toxicity of the chlordane residue present in Great Lakes lake trout and compared the toxicity with the technical mixture and mixtures of pure components.

MATERIALS AND METHODS

Sampling

Lake trout (Salvelinus namaycush) were sampled from southern Lake Michigan near Saugatuck, MI. in conjunction with the fall lamprey predation surveys conducted by the U.S. Fish and Wildlife Service. Approximately 6 to 12 fish, averaging 3.8 kg, were sampled in 1983, 1984, and 1985. Lake trout from Siskiwit Lake on Isle Royale in Lake Superior (average weight 1.2 kg) were sampled in July of 1984 by hook and line. Fish were held on ice until they were frozen and stored at -20°C.

Analytical

Extractions and analyses were performed as described previously (13). Individual fish were partially thawed and a portion of the visceral, adipose rich, muscle (without skin) was removed and immediately processed. A sample of the dorsal muscle was also removed and refrozen. Residues from these tissues are referred to as belly (B) or fillet (F) in the data tables. Developing eggs (E) were analyzed from a subset of females sampled in 1985. Tissues were ground in the presence of 4-7 volumes of anhydrous sodium sulfate using a mortar and pestle. Homogenized tissues were column extracted with methylene chloride, lipids removed via gel permeation chromatography, and chlorinated residues fractionated on florisil, silica gel and charcoal columns.

The methods through the silica gel column are similar to those of Ribick et al. (14). Chlordane components were separated from toxaphene components using a charcoal column (15) as described in detail in Gooch and Matsumura (13). This method involves utilization of a charcoal/Celite® mixture and elution with diethyl ether (chlordane fraction) and benzene (toxaphene fraction).

Chlordane components were quantitated via capillary gas chromatography with electron capture detection using reference standards of chlordane components obtained as a gift from the Velsicol Chemical Co. Concentrations of chlordane components were evaluated twice. The first quantitation was performed on silica gel column fractions from individual fish from different years and locations. Since it was not clear what changes in composition might result from the charcoal column procedure, this quantitation was done to maximize our ability to make comparisons to literature data. In order to conduct toxicity studies, chlordane residues from several fish were pooled, separated from toxaphene components using charcoal column chromatography (13), and requantitated. Recovery studies were not performed for chlordane, however, recoveries for toxaphene were generally greater than 80%. The final chlordane residue was qualitatively confirmed by electron capture negative chemical ionization GC-MS using conditions similar to those described by Stemmler and Hites (16).

Toxicity

Acute bioassays were performed using mosquito larvae as described in Gooch and Matsumura (13). Briefly, 3rd and 4th instar Aedes egypti mosquito larvae (Rockefeller strain) were distributed in disposable culture tubes containing 5 ml of water. Residues were delivered to the tubes dissolved in ethanol. Controls received ethanol at levels equal to the highest amount given to any treatment group. Control mortality was not significant. Acute toxicities, expressed as the 48 hr LC50, were determined using probit regression. Since the distribution of components in the residue is different from the those in the technical mixture, we normalized exposure concentrations for bioassays on the basis of the sum of cis- and trans-chlordane in each test mixture. This approach allows any changes in toxicity that are due to changes in relative amounts of other components to be reflected directly in the acute toxicity value.

The specificity of the biological activity of the residues was evaluated by examining their ability to exhibit a particular pharmacologic property (17, 30). The GABA receptor chloride channel complex contains numerous pharmacologic binding sites (29). One of these binding sites is near the chloride channel and has been shown to to have a high affinity for chlorinated cyclodiene insecticides (12, 17, 18). Binding at this site inhibits chloride flux and thereby prevents the GABA stimulated hyperpolarization of the membrane which characterizes this neuroinhibitory system. It is now believed that chlorinated cyclodiene insecticides exert acute toxicity primarily via this

mechanism (30). In these experiments, we examined the ability of the residues to compete with a radiolabelled chloride channel ligand in a competitive ligand binding assay. The competitive displacement of [^{35}S]-t-butylbicyclophophorothionate ([^{35}S]-TBPS) from its binding site at the chloride channel was determined essentially as described by Abalis et al. (17) and described by us previously (13). The ability to displace this radioligand from its binding site is characteristic of cyclodiene insecticides and is highly correlated with toxicity (12, 17, 18, 30). We used isolated synaptic membranes from the central nervous system of rat and lake trout. Isolation of synaptic membranes from both rat (cerebrum and cerebellum) and lake trout (whole brain) was as described in detail in Gooch and Matsumura (13). We conducted preliminary experiments using technical chlordane to the validate that the binding inhibition was concentration dependent and in the range reported in the literature.

Statistical analyses were done using the Statgraphics® software package.

RESULTS AND DISCUSSION

Residues

Individual fish were analyzed for concentrations of five of the major components of the chlordane residue (though cis-nonachlor was also a prominent component, we did not have the appropriate quantitation standard)(Table 1). Concentrations of the sum of these five components in the adipose rich belly flap from fish from Lake Michigan ranged from 1.2-2.0 μg/g (wet weight). The dorsal fillet contained three to five times less chlordane (as expected based on the lower lipid content) and there was no apparent shift in the relative composition of the components. Lake trout from Siskiwit Lake were approximately three times smaller than those sampled from Lake Michigan and chlordane concentrations were approximately ten times lower. There was a greater differential between the dorsal fillet concentrations and the belly flap concentrations in Siskiwit Lake fish than in fish from Lake Michigan (nearly ten-fold versus approximately five-fold). Since we did not normalize to lipid concentrations, we cannot say whether this is simply a function of the relative lipid enrichment of the tissues analyzed. The concentrations reported here for the Lake Michigan lake trout are similar to those reported by others for fish from the Great Lakes (3, 19). Though this is a limited sample size, there was no apparent decline in total chlordane residues from 1983-1985; an observation which is consistent with the decreasing year to year declines observed for other organochlorine contaminants in lake trout (20).

As expected, the relative contribution of the various components to the residue was substantially different than in technical chlordane. There is a shift in the relative distribution of cis-:trans-chlordane from 1.0:1.2 in the technical mixture (10) to nearly 2:1 in the residue. This

Table 1. Concentration of five major components of chlordane residues in Lake Michigan and Siskiwit Lake lake trout. Concentrations are expressed as μg/g, wet weight, for the belly flap samples and ng/g for the fillet samples (x ± s.d.).

	Heptachlor epoxide	Oxy-chlordane	trans-(γ) chlordane	cis-(α) chlordane	trans-nonachlor	Total[1]
1983						
Belly(5)[2]	0.10 ± 0.03	0.18 ±0.04	0.16 ± 0.06	0.39 ± 0.11	0.39 ±0.11	1.20 ± 0.17
Fillet(3)	14.4 ± 8.8	32.1 ± 15.1	33.7 ± 21.1	74.9 ± 43.5	100.5 ± 42.2	255.6 ± 74.8
1984						
Belly(10)	0.08 ± 0.04	0.27 ± 0.20	0.16 ± 0.07	0.43 ± 0.24	0.41 ± 0.12	1.35 ± 0.16
Fillet(9)	30.4 ± 22.0	61.6 ± 44.4	49.9 ± 26.7	119.6 ± 43.5	180.5 ± 57.2	442.0 ± 61.4
1985						
Belly(6)	0.12 ± 0.11	0.36 ± 0.23	0.26 ± 0.18	0.51 ± 0.28	0.74 ± 0.33	2.00 ± 0.45
Fillet(9)	39.4 ± 45.5	94.7 ± 66.1	73.4 ± 54.5	146.3 ± 91.1	242.2 ± 133.0	595.9 ± 127.2
Siskiwit Lake (Isle Royale, Lake Superior)						
Belly(6)	9.1 ± 5.23	8.4 ± 4.8	13.1 ± 7.1	30.3 ± 16.4	61.2 ± 43.6	122.1 ± 30.0
Fillet(2)	0.8	0.9	2.0	4.7	9.0	17.4

[1] x ± s.e.
[2] Sample size in parentheses
[3] All concentrations as ng/g wet weight

may be toxicologically significant since the cis- isomer is more toxic than the trans- to aquatic organisms (26). The higher proportional abundance of cis-chlordane is most likely due to a less abundant conversion to oxychlordane relative to trans-chlordane (25), which in turn leads to a longer pharmacokinetic half-life (27). Alternatively, Hoff and Chan (28) have recently shown that the profile of chlordane components in the atmosphere over northwest Canada is enriched in cis-chlordane relative to trans- and therefore some of the changes in the ratio of cis-/trans-chlordane observed in residues in fish may be due to processes which occur prior to deposition in aquatic systems. Interestingly, there was no apparent difference in the qualitative composition of the residue between Lake Michigan and Siskiwit Lake fish. The chlordane residues in lake trout from Siskiwit Lake on Isle Royale in Lake Superior have also been reported by Swackhamer and Hites (22) and are derived only from an atmospheric source (21). It is logical to assume that Lake Michigan receives chlordane from a mixture of atmospheric and surface sources, though it is difficult to partition their relative importance. Despite this potential difference in source terms, the relative abundance of the chlordane components appearing as residues in lake trout from the two different systems is similar. This similarity, combined with the strong presence of the metabolic products heptachlor-epoxide and oxychlordane, suggests that the primary determinant of the relative abundance of components in the residue is driven by metabolic processes occurring in the fish. The relative abundance of components in fish seen here has also been reported by others (11, 23).

To obtain sufficient material to conduct acute toxicity bioassays, residues from individual fish were pooled and purified from toxaphene and DDT related compounds via charcoal column chromatography. Representative GC-ECD traces of technical chlordane and of a purified chlordane residue from lake trout are shown in Figure 1 along with a representative GC/MS (EC-NCI) mass chromatogram.

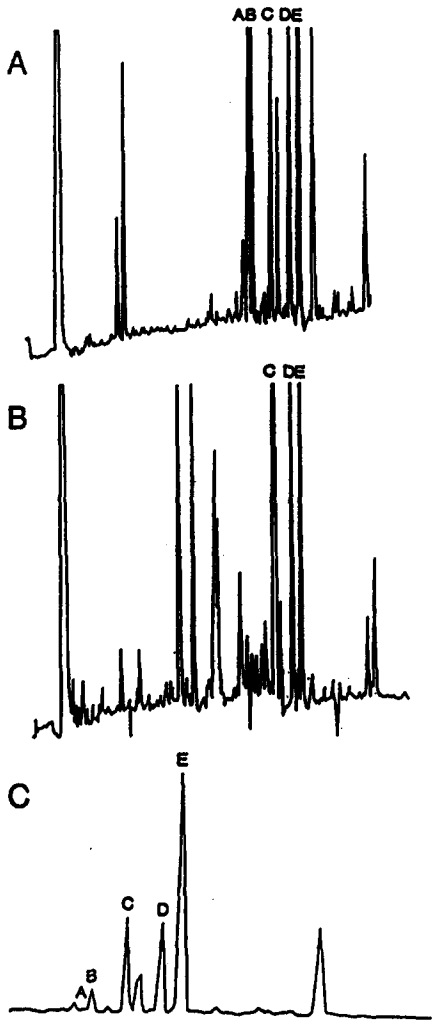

Figure 1. Capillary gas chromatograms of (A) chlordane components isolated from lake trout (ECD); (B) technical chlordane (ECD); and (C) chlordane components isolated from lake trout (EC-NCIMS). A-heptachlor epoxide, B-oxychlordane, C-cis-chlordane, D-trans-chlordane, E-trans-nonachlor.

The mass chromatogram confirms that these residues were highly purified; only minor amounts of residual toxaphene components were detected (not shown). A substantial portion of cis-nonachlor eluted with the toxaphene components and hence its contribution to the purified residue was much less than in the semi-purified extract. The apparent decrease in the abundance of heptachlor

epoxide in the EC-NCI chromatogram appears to be due to a lower response factor for heptachlor epoxide relative to oxychlordane than obtained with the electron capture detector.

Acute Toxicity

In order to simplify comparison of samples used for toxicity studies, we have listed the relative abundance of only four components of chlordane residues (Table 2).

Table 2. Relative composition of pooled chlordane residues isolated from Great Lakes lake trout.

Residue[1]	Component (% of sum)			
	HE	Oxy	cis-	trans-
83B	18.1	25.4	20.3	36.2
83F	12.4	22.4	22.2	43.0
84B	18.3	26.1	19.0	36.6
84F	14.7	32.2	16.9	36.2
85B	14.4	23.3	24.5	37.8
85F	15.0	26.4	17.5	41.1
85E	13.6	28.6	17.2	40.7
SISKL	14.5	19.4	23.4	42.7
HEOCT[2]	15	25	21	39

[1] Year and tissue.
[2] HEOCT is the mixture of pure standards of heptachlor epoxide, oxychlordane, cis-chlordane and trans-chlordane.

We have not included trans-nonachlor since studies by Ivie et al. (24) and Tashiro and Matsumura (25) have shown that the most toxic components of chlordane are cis- and trans-chlordane or the metabolites oxychlordane and heptachlor epoxide. These literature data and the data which follow support this presentation. Cis- and trans-chlordane comprise 55-65% of the sum of these four components, oxychlordane 19-30%, and heptachlor epoxide 12-18%. As stated previously, heptachlor epoxide and oxychlordane are products of oxidative metabolism and presumably arise via metabolism in the lake trout or some other part of the biotic system.

The acute toxicity of the purified chlordane residue was determined using an acute bioassay with mosquito larvae. All test concentrations were normalized to the sum of cis- and trans-chlordane. Normalizing the exposures on the basis of the sum of cis- and trans-chlordane allows changes in toxicity which are due to changes in the ratio of cis-to trans-chlordane and/or due to the presence of additional components to be reflected directly in the acute toxicity value. The purified

chlordane residues from tissues of Lake Michigan lake trout were 3-6 times more toxic than technical chlordane (Table 3).

Table 3. Toxicity of purified chlordane residues to Aedes egypti mosquito larvae. Forty-eight hour LC-50 values in µg/l were calculated on data pooled from two experiments except where noted by *.

Residue[1]	48 hour LC50 (95% C.I.)	Slope[2]
83B*	23.1 (18.8-27.4)	4.4
84B	26.0 (22.8-29.5)	4.2
85B	53.4 (49.5-58.4)	7.2
84F	40.2 (35.2-46.5)	3.4
85F	32.9 (27.5-37.2)	4.1
85E*	41.9 (33.8-55.9)	3.5
Tech. Chlordane	147.2 (121.1-167.2)	4.0
cis:trans (1:1)	225.9 (192.8-269.3)	3.6
HEOCT	46.0 (38.0-53.5)	5.2

[1] See Table 2 for description.
[2] Slope of the probit regression.

Chlordane residues were also 5-10 fold more toxic than a 1:1 mixture of cis- and trans-chlordane, indicating that components other than cis- and trans-chlordane add to the toxicity in both the residues and the technical mixture. There was no difference in the toxicity of residues among tissues or years, a result consistent with the qualitative similarity among samples. Though we did not have a sufficient quantity of material to test the acute toxicity of residues from Siskiwit Lake fish, the composition of the residue strongly suggests that the toxicity would be similar to the residues from Lake Michigan fish. The data on neural target site binding affinity which follows strongly supports this hypothesis.

As stated above, we hypothesized from the literature available on the toxicity of individual chlordane components (12) that the components of concern for acute toxicity were heptachlor epoxide, oxychlordane, and cis- and trans-chlordane. To investigate the cause for the increased toxicity of the chlordane residue, we constructed a mixture of pure standards of these four components (HEOCT), at relative proportions which approximated the residues found in the lake trout. As shown in Table 3, HEOCT was much more toxic than technical chlordane and its toxicity was similar to the residues. Since the mixture of 1:1 pure cis- and trans-chlordane was much less toxic than the residues, simply enriching the relative amount of cis-chlordane does not

appear to enhance toxicity in this bioassay. The increased toxicity of the residues appears to be mainly due to the presence of the metabolites, heptachlor epoxide and oxychlordane. Lawrence and Casida (12) tested the acute toxicity of these four components to mice and found a rank order of toxicity of heptachlor epoxide>oxychlordane>cis-chlordane>trans-chlordane. It is important to note, and perhaps not surprising, that the mixture of standards successfully reproduced the toxicity of the residue from lake trout without including trans-nonachlor or any other chlordane components that are in the residue (e.g. the chlordane isomer eluting between cis-and trans-chlordane (11)), in the test mixture.

Binding Studies

In order to validate the specificity of the toxicity of the residues (i.e. that the toxicity observed was due to chlordane and not something else), we utilized our knowledge of the mechanism of action of these compounds at the presumptive neural target site. We demonstrated the utility of this approach for testing purified toxaphene residues isolated from this same series of samples in a previous report (13). In these experiments, we examined the interaction of the chlordane residues with the chloride channel in rat and lake trout brain. We chose to work with rat brain because it is a well-characterized system and provided a means of validating the technique for this study. Though less well characterized, the GABA receptor in fish appears to be similar to the one in mammals (31, 32). Also, fish are exquisitely sensitive to some cyclodienes and we wished to make comparisons of the toxicologic potency in rat versus lake trout brain.

In preliminary experiments (not shown) we obtained fifty percent inhibition of [^{35}]-TBPS binding at a total cis- + trans-chlordane concentration of approximately 7.8 x 10^{-8} M. This potency is well within the range established as significant for correlating toxicity within this target site (17). We utilized this concentration for point estimates of the inhibitory properties of the residues. The results of the competitive binding studies are presented as the percent inhibition of the binding of [^{35}S]-TBPS in Table 4. As with the acute bioassay, the residues from lake trout were much more effective inhibitors of [^{35}S]-TBPS binding than either the technical standard or a 1:1 mixture of pure cis- and trans-chlordane. Increasing the ratio of cis-:trans-chlordane to 3:2 (close to what it is in the residue) increased the inhibition of binding, but not enough to explain the inhibitory potency of the residues. As with the acute toxicity, the mixture of chlordane standards (HEOCT) was much more inhibitory than the technical mixture and was similar to the residues. Furthermore, as expected from the mechanistic hypothesis, the degree of inhibition of the binding of [^{35}S]-TBPS in both rat and lake trout brain was closely correlated with the acute toxicity (Figure 2a). In addition, there was a close correlation between the degree of inhibition in the lake trout and rat brain preparations (Figure 2b); they yielded essentially the same results. This is strong evidence that the toxicity we observed is specific to chlordane and not to unidentified

Table 4. Inhibition of [^{35}S]-TBPS binding to synaptic membranes from rat and lake trout brain by chlordane residues isolated from Great Lakes lake trout.

Residue	% Inhibition Rat Brain[1]	% Inhibition Lake Trout Brain[2]	Acute Toxicity[3]
83B	78.3	76.4	23.1
84B	79.6	76.3	26.0
85B	66.5	62.6	53.4
83F	58.4	47.5	-ND-
84F	73.3	70.3	40.2
85F	75.3	75.3	32.9
85E	69.4	70.2	41.9
SISKL	66.2	60.7	-ND-
Tech Chlor	49.8	47.7	147.2
cis:trans (2:3)	39.3	-ND-	-ND-
cis:trans (1:1)	36.1	-ND-	225.9
cis:trans (3:2)	43.8	-ND-	-ND-
HEOCT	70.8	67.6	46.0

[1] x of two experiments.
[2] x of four experiments.
[3] 48 hour LC-50 in ug/l.
[4] Value estimated from concentration response curve.

components of the purified residue. We suggest that the variation in the toxicity we observed among residues is due primarily to variations in the pooling and the preparation of the residues rather than intrinsic differences of the native residues.

Since acute toxicity and inhibition of [^{35}S]-TBPS binding did not vary consistently among years or tissue types and we did encounter differences in toxicity among samples, we sought to determine if toxicity varied in a manner consistent with the relative composition of the test mixtures. Our experiments clearly suggest that the enhanced toxicity of the residues is due to the presence of heptachlor epoxide and oxychlordane, though they do not suggest one or the other other, specifically. We used simple regression analysis to further clarify the relationship between the relative proportion of specific components and toxicity. To look at the influence of individual variables we regressed toxicity and percent inhibition of [^{35}S]-TBPS binding as a function of the relative abundance of each of the components. The relative abundance of heptachlor epoxide in the residue was significantly correlated to both toxicity and inhibition of [^{35}S]-TBPS binding (Table 5). This correlation suggests that the variability in the response among samples was due

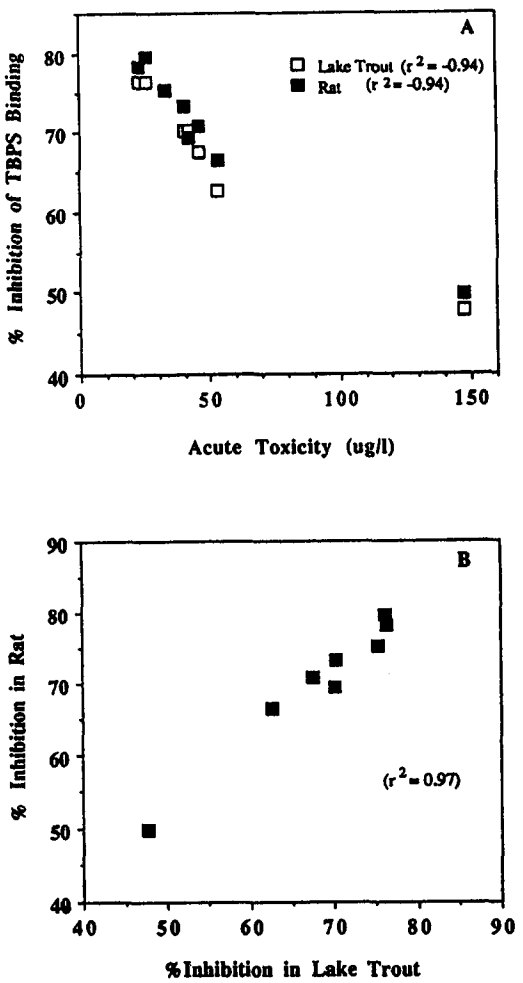

Figure 2. (A) Correlation between inhibition of [^{35}S]-TBPS binding in rat and lake trout brain preparations and acute toxicity. (B) Correlation between inhibition of [^{35}S]-TBPS binding by chlordane residues in rat and lake trout brain preparations.

primarily to varying quantities of heptachlor epoxide. This result is consistent with the studies of chlordane toxicity in birds (33), which showed a significant correlation between mortality and the level of accumulated heptachlor epoxide in the brain. This analysis does not, however, indicate that the increased toxicity of the residue is due only to heptachlor epoxide. Lawrence and Casida (12) have shown that heptachlor epoxide and oxychlordane have similar potencies in the [^{35}S]-TBPS binding assay and both are more toxic and more effective inhibitors of [^{35}S]-TBPS binding than either cis- or trans-chlordane.

Table 5. Correlation coefficients for toxicity and binding to the neuromolecular target site versus the relative proportions of the various components of the pooled chlordane residues.

Component	Toxicity[1]	$P < \alpha$	Binding[2]	$P < \alpha$
Heptachlor epoxide	-0.82	0.023	0.88	0.002
Oxychlordane	-0.10	0.84	0.50	0.17
trans-chlordane	0.44	0.32	-0.57	0.11
cis-chlordane	0.29	0.52	-0.71	0.03

[1]Toxicologically significant correlations are negative.
[2]Toxicologically significant correlations are positive.
[3]Toxicity regressions have 6 d.f. and binding regressions have 8 d.f.

CONCLUSIONS

Chlordane residues in Lake Michigan and Siskiwit Lake lake trout are significantly more toxic than the chlordane that was used in agricultural and domestic applications. Our studies and the available information in the literature suggest that the increased toxicity is due to the significant abundance of the stable metabolites heptachlor epoxide and oxychlordane in the residue, though the variation in the toxicity of the different residues we studied was correlated with the relative abundance of heptachlor epoxide. The relatively high abundance of cis- and trans-nonachlor does not appear to play any role in the acute toxicity of the residue. Our results emphasize the need to understand the toxicology of the residues of complex mixtures in relationship to the technical product. Further studies are necessary to determine if other properties associated with chlordane, such as carcinogenicity, are influenced to the same degree as acute toxicity.

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