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With regards JS

Maternal Transfer of Chlordane and Its Metabolites to the Eggs of a Stream Mayfly *Centroptilum triangulifer*

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Mayflies transferred ~70% of their chlordane load into eggs along with 44–52% of their lipids. Lipid pools comprising adult tissues and their eggs were not at equilibrium in terms of chlordane concentration, with the eggs containing several times that of the mothers' adult tissues. However, chlordane fingerprints in eggs and adults were similar in terms of distribution of chlordane components and metabolites. Mayflies fed pretreated algae were indistinguishable from those exposed to chlordane added to their water. Mayflies transformed the technical chlordane into a signature dominated by the nonachloro component *trans*-nonachlor and the two metabolites heptachlor epoxide and oxychlordane. This was anomalous in comparison to organisms of similar trophic levels and more like the level of transformation measured in higher organisms, such as the porpoise and humans. Disequilibrium between lipid tissues of mayflies and their eggs highlights the limitations of modeling contaminant impact by measuring only whole body contaminant burdens.

Introduction

Maternal transfer of nonpolar organic contaminants to offspring has been documented for numerous species such as fish, lobsters, chickens, and humans (*1–7*). The biological implications of such transfer include toxicity to offspring, shifts in expected route of food chain transfer, and intergenerational accumulation of contaminants. The mechanisms of contaminant transfer for egg-producing organisms include incorporation into the lipids forming the egg mass, passive diffusion into formed eggs, and active transport into eggs via "vitellogellin" transfer (*7*). For mammals, the processes include transplacental transport (*3*) and transfer of lipophilic contaminants in milk fat (*8, 9*). For females of certain organisms such as fish, seals, and humans, maternal transfer to offspring is substantial, and data suggest that resulting contaminant burdens are lower than those of corresponding males (*7, 8*).

In clean streams, eggs have already been demonstrated to be a primary source of organic contaminants for trout feeding on eggs of migrating salmon (*10*). Because the mayfly, *Centroptilum triangulifer* (formerly *Cloeon triangulifer* McDunnough), uses approximately 50% of her body lipids to produce eggs, it seems reasonable to hypothesize that an equivalent portion of lipid-soluble contaminants would also be transferred. Mayflies, which are a dominant group of stream-dwelling macroinvertebrates, die soon after laying their eggs. Thus, the impact of maternal transfer of contaminants for mayflies is primarily limited to the toxicity to their offspring and the use of larvae and adults as food by other organisms. While mayfly eggs and their newly hatched larvae may appear to be insignificant in the food chain due to their size (<1 μg dry weight) (*11*), they are abundant and may provide food for meiofauna, oligochaetes, or other macroinvertebrates which filter feed or process sedimentary materials for food. Also, mature mayfly larvae, which contain a full complement of eggs, are readily fed on by fish as they crawl or swim to the stream surface or edge to molt to the winged adult (subimago). Adult mayflies are also preyed on by fish when they return to oviposit in the stream as well as by birds, bats, and other terrestrial predators that feed on flying insects (*12*).

The primary goal of our study was to measure the extent of maternal transfer of organic contaminants by mayflies to their eggs. We used technical chlordane as our model contaminant for the following reasons: (1) although banned for use in the United States since 1987, it is a persistent nonpolar pesticide and is widely distributed in organisms throughout the environment (*13–18*); (2) previous work on the toxicity of technical chlordane to mayflies (*19*) provided information on the maximum sublethal concentration usable such that detectable residues in the samples could be obtained without significant mortality of the experimental organisms; and (3) technical chlordane is comprised of a mixture of compounds differing in susceptibility to bioaccumulation and metabolism by organisms representing different trophic levels in the food chain. This allows a tracking of the contaminant transfer processes within the organism and between trophic levels through analysis of both the concentration of chlordanes and the composition of the chlordane fingerprints reflecting varying degrees of transformation.

Unaltered technical chlordane is a mixture of over 140 chlorinated cyclic compounds dominated by compounds containing seven (heptachlor), eight (*trans-* and *cis-*chlordane), and nine chlorines (*trans-* and *cis*-nonachlor, nonachlor III) (*20–22*). The metabolites heptachlor epoxide and oxychlordane are not found in the technical mixture used for pest control. Heptachlor and *trans-* and *cis*-chlordane can be metabolized to heptachlor epoxide and oxychlordane, respectively (*23–25*). Some organisms are also capable of transforming *trans*-nonachlor to *trans*-chlordane and thus further to oxychlordane (*25*). Generally, the higher trophic level organisms transform the chlordane mixture more thoroughly (*16, 17*).

Materials and Methods

Rearing, Exposure, and Collection of Mayflies and Eggs. The study mayfly *C. triangulifer* inhabits streams and is parthenogenetic (thelytokous type), reproducing as clones (*19*). Eggs were collected from one clone (clone 89–2) isolated from White Clay Creek (Chester County, Pennsylvania). They were hatched in small jars (50 mL) containing streamwater and reared in glass vessels (1.8 L) as discussed in more detail elsewhere (*19*). All exposure experiments, except when noted otherwise, were carried

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out in filtered streamwater at 20 °C (White Clay Creek, Chester County, Pennsylvania). Briefly, 50 newly hatched first instar mayfly larvae were transferred to each of the 1.8-L glass jars containing an air stone and an acrylic plate (6.5 × 23 cm) supporting ~5 g of algae (wet weight, primarily diatom species).

Larvae were reared to the adult stage (about 4–8 weeks) while exposed to one of four treatments in triplicate: (a) methanol (HPLC grade, Burdick and Jackson) controls, 100 μL/1.5 L; (b) 1.5 L of 4.53 μg/L technical chlordane (Accustandard, New Haven, CT, in 100 μL of methanol carrier); and (c) and (d) algal plates transferred to larval rearing jars after exposure for 48 h to 1.5 L of streamwater containing 4.53 or 45.3 μg/L technical chlordane, respectively (concentrations of chlordane represent values measured at the start of the exposure experiment). In the latter experiments, algal plates were replaced periodically with fresh plates containing algae also exposed for 48 h to a given level of chlordane in streamwater. Overall, four algal plates were added over the span of the larval growth period in all experiments. The water in jars containing mayfly larvae exposed directly to chlordane (experimental treatment b) was replaced periodically with 1 L of fresh streamwater containing 6.8 μg/L chlordane in 100 μL of methanol carrier (this gave a final concentration of 4.53 μg/L for 1.5 L). This plus the addition of non-chlordane-exposed algal plates occurred simultaneously with plate changing in the other exposure jars. Exposure of mayflies to chlordane thus occurred from newly hatched larvae to adult emergence.

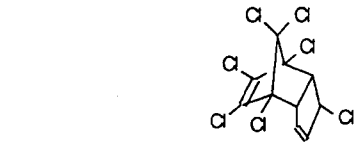
Emerging winged mayflies (subimagos) were enumerated and after molting (~1 day later) to the imago or true adult were placed in 50-mL organically cleaned jars containing 20 mL of nanopure water (Millipore) and allowed to oviposit. Spent adults were then transferred to a 4-mL amber vial with Teflon cap liners. The first 10 emerging mayflies from each exposure vessel or their eggs were composited to provide one sample each of eggs or adults for chlordane component analysis. Eggs from the next two mayflies to emerge from each exposure vessel were incubated at 20 °C, and 50 hatchlings (F_1) from each clutch were reared to adulthood to determine F_1 larval survivorship. Subsequent groups of 10 emergent mayflies and their eggs were collected as composites for chlordane component analysis. Survivorship of the first generation in the d treatment (45.3 μg/L of algae) was too low to obtain sufficient replication for studies on the second generation.

Larval survivorship (%) for each generation reflected the proportion of larvae that successfully emerged as winged adults in each rearing vessel. The duration of larval development represented the total number of days required by each individual to go from a newly hatched larva to the winged adult.

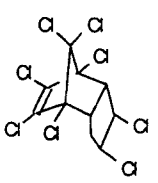
Extraction and Analysis. Adults were kept frozen, and the eggs were refrigerated until analysis. All egg samples were extracted within 1 month to prevent embryonic development from altering fat or chlordane content. Adult mayflies were spiked with 2,2′,3,4,4′,5,6,6′-octachlorobiphenyl (IUPAC No. PCB204) as a surrogate standard and ground using a tissue homogenizer with a 1:1 mixture of methylene chloride–methanol. Eggs were more difficult to pulverize, requiring the use of a conical vial and a roughened glass rod as mortar and pestle and the removal of as much water as possible from the eggs

Chart 1

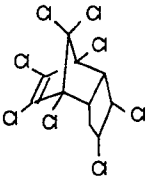
COMPONENTS OF TECHNICAL CHLORDANE



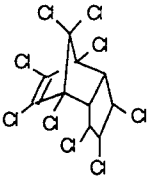
HEPTACHLOR



cis-CHLORDANE

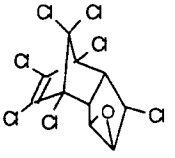


trans- CHLORDANE

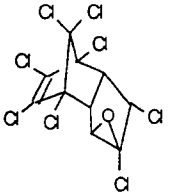


trans- NONACHLOR

METABOLITES OF TECHNICAL CHLORDANE



HEPTACHLOREPOXIDE



OXYCHLORDANE

prior to grinding. PCB204 was also added to the eggs prior to extraction as a surrogate standard. Homogenized adult mayflies and eggs were sonicated (sonic bath) with two 5-mL aliquots of 1:1 methylene chloride–methanol for 10 min/aliquot. Extracts were filtered and exchanged to hexane by rotary evaporation. Extract weights (i.e., lipid content) were determined by weight after drying one-tenth of the hexane-dissolved extract on a small preweighed aluminum foil boat. Chlordane components were separated from lipids using microcolumns of 1 g of 1% (w/w) nanopure water-deactivated silica gel. The fraction containing technical chlordane components and metabolites was eluted with 15% methylene chloride in hexane and concentrated using rotary evaporation and nitrogen blowdown for the final phase.

Concentrated mayfly and egg extracts (10–20 μL total volume) were analyzed by gas chromatography/mass spectrometry for four major chlordane components, heptachlor (HEP), *trans*- and *cis*-chlordane (tCHL and cCHL, respectively), and *trans*-nonachlor (tNONA), and the two metabolites heptachlor epoxide (HPX) and oxychlordane (OXY) (see Chart 1 for structures). Volumes of 1 μL of extracts were co-injected with octafluoronaphthalene as an internal standard onto a 0.25-μm coating thickness, 0.25-mm i.d., 30-m DB-5 (J&W Scientific) fused-silica capillary column and separated using a temperature program of 60 °C (held 2 min), ramped to 200 °C at 20 °C/min, ramped to 230 °C at 2 °C/min, ramped to 280 °C at 20 °C/min, and held at 280 °C for 3 min. Components were identified and quantified using a Hewlett Packard

Table 1. Concentration of Chlordane Components in Mayflies and Their Eggs [ng/g of lipid, Mean (SD)][a]

treatment	lifestage	no. of samples[b]	HEP	tCHL	cCHL	tNONA	HPX	OXY	ΣCHL
methanol controls	adults	7	bdl	bdl	bdl	bdl	bdl	bdl	bdl
	eggs		bdl	0.4 (1.1)	bdl	7 (19)	bdl	bdl	8 (19)
4.5 μg/L in water	adults	9	bdl	bdl	bdl	470 (350)	bdl	14 (17)	480 (360)
	eggs		bdl	12 (25)	7 (21)	1000 (700)	180 (290)	63 (50)	1300 (750)
4.5 μg/L through algae	adults	10	bdl	0.1 (0.3)	0.1 (0.3)	310 (310)	6 (12)	42 (59)	360 (330)
	eggs		bdl	9 (15)	4 (7)	620 (810)	230 (450)	110 (100)	940 (1300)

[a] Abbreviations: bdl, below detection limit; HEP, heptachlor; tCHL, *trans*-chlordane; cCHL, *cis*-chlordane; tNONA, *trans*-nonachlor;; HPX, heptachlor epoxide; OXY, oxychlordane; ΣCHL, sum of chlordanes, including the metabolites. [b] Each sample represents composites of 10 individuals or egg clutches or remainder of hatch.

5988, RTE-A, quadrupole mass spectrometer, with selected ion monitoring (SIM) and electron capture negative ionization (ECNI, 0.45 Torr CH_4) to improve sensitivity and selectivity. Several standards were run prior to each suite of samples, with a check standard following each set of 10 analyses. Because the calibration curves were not linear, quantitation of peaks was carried out by comparison of the response factor of the next highest standard based on the area of the peak.

Statistical analyses of the differences between chlordane content of eggs and their mothers were performed using a Wilcoxon signed-rank test (SAS Institute Inc., Cary, NC). Below detection limit values were calculated as zeros, in spite of the potential for skewing of results. We considered the use of an arbitrary value such as one-half the detection limit for calculations; however, while a mid-range value might be appropriate for exposed mayflies, this would probably not be the case for control samples and for compounds rarely detected. With subsets of data represented by significantly different distributions of data points below the detection limit, we ruled out the use of a mid-detection limit value for calculating results.

Results

Emergence and Viability. Chlordane-exposed, first generation larval mayflies averaged 66% (SD 25%) and 58% (SD 30%) survivorship for treatment c (4.5 μg/L, algae) and treatment b (4.5 μg/L, water), respectively. These values were not significantly different from average survivorship in the control (62%, SD 35%, *t*-test, $p < 0.05$). Average larval survivorship of the second generation was also indistinguishable between controls and chlordane-exposed mayflies [82% (SD 12%) emergence for second generation controls versus 72% (SD 28%, 4.5 μg/L, algae) and 82% (SD 10%, 4.5 μg/L, water) emergence for second generation of chlordane-exposed mayflies, *t*-test, $p < 0.05$]. The duration of the larval period for second generation mayflies was approximately the same for all treatments [33.6 d (SD 1.6 d, 4.5 μg/L, algae) and 32.2 d (SD 2.3 d, 4.5 μg/L, water) versus 31.6 d (SD 1.5 d, controls), *t*-test, $p < 0.05$]. Effects on subsequent generations or susceptibility to toxicity of chlordane by the second generation were not tested.

Lipid content was measured for adults and their eggs. Average lipid content of chlordane-exposed adults was 0.19 (SD 0.03, 4.5 μg/L, algae) and 0.18 (SD 0.07, 4.5 μg/L, water) mg/individual. Lipid content of control adults [0.13 mg (SD 0.01 mg)/individual] was only significantly lower than that of mayflies exposed to chlordane-treated algae (*t*-test, $p < 0.05$). Lipid content did not differ significantly between controls and mayflies exposed to chlordane-treated water (*t*-test, $p < 0.05$). Eggs from all three treatments were similar at 0.16 (SD 0.05, 4.5 μg/L, algae), 0.14 (SD 0.03, 4.5 μg/L, water), and 0.14 (SD 0.04, control) mg of lipid/clutch (*t*-test, $p < 0.05$). In general, the study mayflies deposited 44–52% of their total lipid content into their eggs, with exposed adults containing slightly more lipid than controls (*t*-test, $p < 0.05$). This is probably due to the slightly larger size and longer larval life stage of mayflies exposed to chlordane (*19*).

Chlordane Content of Mayflies and Their Eggs. Mayfly and egg extracts were analyzed for six chlordane components (see Chart 1 for structures). Four of the compounds were present in the original technical chlordane used in the experiments and included heptachlor (HEP), *trans*- and *cis*-chlordane (tCHL and cCHL, respectively), and *trans*-nonachlor (tNONA). We also analyzed the extracts for the two metabolites heptachlor epoxide (HPX), a metabolite of heptachlor, and oxychlordane (OXY), a metabolite of *trans*- and *cis*-chlordane. Oxychlordane can be produced by some organisms after the transformation of *trans*-nonachlor to *trans*-chlordane (*23–25*). Results are presented in Table 1 as part per billion (ng/g) normalized to lipid content because egg wet weights were not measured, and this measure allows the direct comparative analysis of the transfer of lipid-soluble contaminants to the offspring by mayflies. As noted earlier, mayflies were exposed to technical chlordane by direct addition to water or through the feeding of algae previously exposed to chlordane. However, results from the two experiments were statistically indistinguishable (Wilcoxon signed-rank test, $p < 0.05$) and, thus, will be combined in presenting the following results.

Heptachlor, a component of technical chlordane containing seven chlorines, was not detected in any of the samples, possibly due to complete metabolism and/or the higher detection limit under the ECNI mass spectral conditions. The chlordane fingerprint consisting of the remaining five compounds in exposed mayflies and their eggs was dominated by tNONA and OXY (see Figure 1). Chlordane components in control adults and eggs were below detection for all components except for 50 ppb tNONA in one egg sample and 3 ppb tCHL in another. This contamination may have originated in the water supply, which was collected from a stream located in a watershed exposed to chlordane in the past. Second generation mayflies (analyzed with eggs) contained non-detectable levels of chlordane, understandable because the mayfly grows by several orders of magnitude during development (*11*), thus diluting the original contaminant load from the egg to below the detection limit. For example, an egg weighing 0.4 μg (dry weight) with 1100 ppb Σchlordanes/lipids grows to 1 mg. It would then

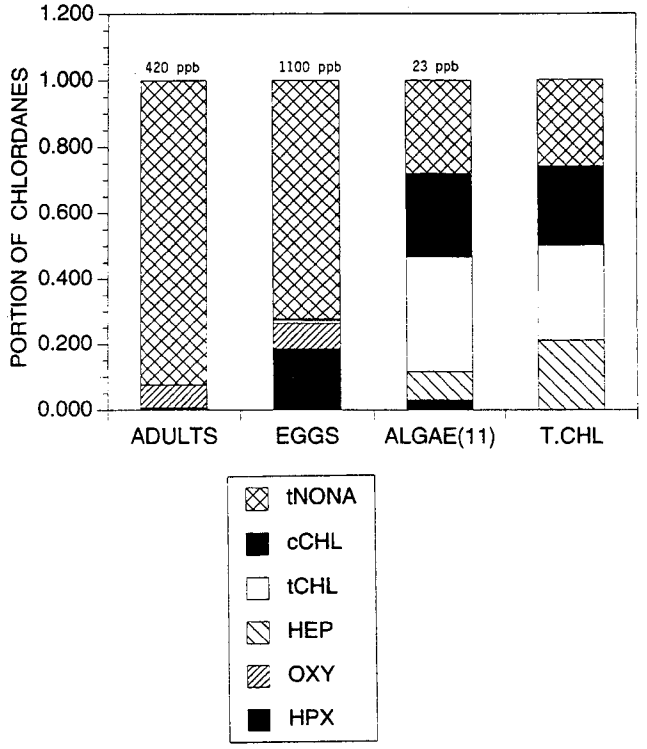


Figure 1. Relative proportion of chlordane components in technical chlordane-exposed mayfly eggs and adults (tCHL and cCHL were 1.0 and 0.5 % for mayfly eggs). The composition of components in technical chlordane (T.CHL) and in algae exposed to 10.7 μg/L of technical chlordane are included for comparison (algae exposed to 4.5 μg/L would contain ~10 ng/g by extrapolation). Abbreviations are as follows: HPX = heptachlor epoxide, OXY = oxychlordane, HEP = heptachlor, tCHL = *trans*-chlordane, cCHL = *cis*-chlordane, and tNONA = *trans*-nonachlor.

contain 0.0004 ppb chlordane transferred by the mother, which would be undetectable by our methods.

The octachloro compounds, tCHL and cCHL, were only present in one chlordane-exposed adult composite at 1 ppb for each compound. The eggs, on the other hand, contained low but more often detectable quantities at 10 (SD 20) and 5 (SD 15) ppb tCHL and cCHL, respectively. Metabolism of the octachloro compounds appeared to be very thorough for all exposed mayflies. The nonachloro component, tNONA, was present at 820 (SD 770) and 390 (SD 330) ppb in exposed eggs and adults, respectively. Metabolites HPX and OXY were also present, the former more often undetectable (25 versus 11 below detection of 38 samples for HPX and OXY, respectively) but otherwise present at high concentrations in the eggs. HPX content in eggs and adults were 210 (SD 380) and 3 (SD 9) ppb, respectively. OXY concentrations were 89 (SD 82) and 29 (SD 45) ppb for eggs and adults, respectively.

Concentrations of OXY, tNONA, and total chlordanes (ΣCHL) were compared in eggs versus adults to monitor the equilibrium of contaminant between the lipid used for egg generation versus other lipid stores in the mayfly. A comparison between eggs and spent adults for t- and cCHL and HPX was difficult because they were often below detection and varied up to two orders of magnitude; however, eggs clearly contained more of these contaminants/mg of lipid than adults. Eggs contained significantly higher [i.e., a factor of 2.1 higher (SD 1.7)] tNONA compared to the corresponding adults ($p < 0.05$, Wilcoxon signed-rank test). Three of the 19 samples were incalculable due to one or both of the values being below detection, primarily in the adult samples. The metabolite OXY was present in eggs at significantly higher concentrations than in adults [i.e., a factor of 4.0 higher (SD 5.0), $p < 0.05$, Wilcoxon signed-rank test; 10 samples were incalculable due to nondetectable values]. Total chlordanes were also significantly higher in eggs than adults [i.e., a factor of 2.7 (SD 2.3) higher, $p < 0.05$, Wilcoxon signed-rank test, three samples incalculable]. While ratios varied substantially, all compounds detected were present in eggs at lipid-normalized concentrations several times those in the corresponding adults.

Discussion

Previous research indicated an increase in larval development time for mayflies exposed directly to technical chlordane (*19*). Although the emergence of the offspring of chlordane-exposed individuals in this study was also slightly later than control mayflies, the time lag was not statistically significant. This resulted in a higher total lipid content for the adults but not the eggs. As will be discussed below, this may account for some of the disequilibrium between the chlordane content of eggs and other adult lipid tissues.

The algae associated with the experiment altered the original technical chlordane composition by converting some HEP to HPX; thus, they produced a signature with relatively enhanced levels of the octa- and nonachloro components, tCHL+cCHL and tNONA, respectively (Figure 1). OXY, primarily the metabolite of the octachloro compounds, was not detected in the algae. However, mayflies significantly altered the chlordane fingerprint by converting most of the hepta- and octachloro components to metabolites and accumulating the nonachloro component, tNONA. Metabolically, this is consistent with the relative ease of enzymatic oxidation of the heptachloro and octachloro components over the nonachloro compounds (*23–25*). The greater number of chlorines apparently blocks access to the carbon bonds for oxidation (see Chart 1) (*26*). The half-life of nonachlor, which is indicative of susceptibility to metabolism and excretion, was determined to be approximately three times that of the octachloro compounds in rats (*26*).

Organisms such as rats are capable of transforming tNONA to tCHL and from there further to OXY, which then accumulates as the metabolic end point (*24*). Chlordane fingerprints in these animals are generally dominated by OXY rather than tNONA. Humans do not have that capability and thus have a chlordane signature dominated by tNONA (*25*). It appears that the mayflies also lack this enzymatic capability, as their chlordane fingerprint was dominated by tNONA, and all the OXY present could be accounted for from the original tCHL and cCHL content.

The chlordane fingerprint for extracts of mayflies and their eggs (data combined) was anomalous when compared with other aquatic species at similar and higher trophic levels. For example, Figure 2 shows the chlordane fingerprints of samples from this study, data of Kawano *et al.* (*16*) for aquatic organisms, and data on human milk from Taguchi and Yakushiji (*9*). Samples from similar trophic levels presented by Kawano *et al.* (*16*) were combined for this comparison. Biologically, the most comparable trophic level for mayflies was that of krill and

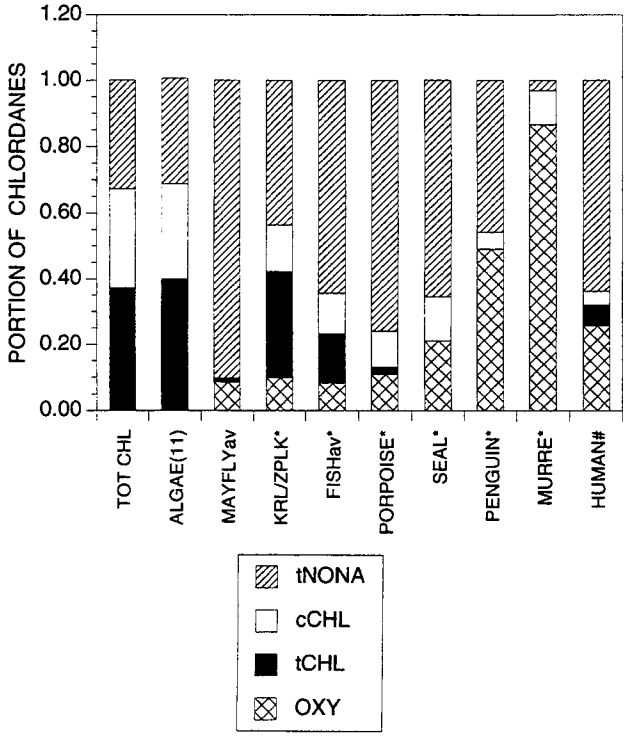


Figure 2. Comparison of the chlordane fingerprint present in mayflies (includes combined data from eggs and adults) from this study with other aquatic organisms from the data of Kawano *et al.* (ref 15, denoted by *) and chlordane content of human milk from the data of Taguchi and Yakushiji (ref 9, denoted by #). Abbreviations are as follows: OXY = oxychlordane, tCHL = *trans*-chlordane, cCHL = *cis*-chlordane, tNONA = *trans*-nonachlor, T.CHL = technical chlordane, KRL/ZPLK = krill/zooplankton, and av = average of data. Heptachlor and heptachlor epoxide were not studied by Kawano *et al.* (*15*) and thus were deleted from the mayfly data for comparison purposes.

zooplankton. However, the mayfly chlordane fingerprint showed a far greater degree of transformation and was dominated by tNONA, retaining little of the octachloro compounds t- and cCHL and none of the heptachloro compound HEP. OXY composition was similar between mayflies and krill/zooplankton, at approximately 10% of the total. Chlordane signatures in mayflies and their eggs were most comparable to the porpoise and seal, with tNONA as the dominant component and OXY contributing 10–20%.

The mayflies also clearly transferred substantial quantities of chlordane components into their eggs. This may have occurred by simple incorporation of chlordane along with the lipids that formed the eggs or may have included components transferred across the egg wall after encapsulation. In any case, the two lipid tissues were not at equilibrium, with the eggs containing several times the chlordane burden as lipid tissues remaining with the mothers. The mayfly ultimately transferred over 70% of her total burden of chlordanes to the eggs. Numerous other researchers have documented maternal transfer of organochlorine contaminants. Chickens transferred 55% of their total burden of radiolabeled methyl mercury to their eggs within 70 days of administration of the dose (*5*). The proportion of lipid-normalized contaminant passed to eggs in other studies ranged from the lobster egg mass, which contained twice the levels of DDT as the remaining carcass and hepatopancreas (*1*), to rainbow trout and white suckers who transferred 11% and 16% of their lipids to their eggs, respectively, but only 6% and 9% of their total chlordane burden (*2*). Perch and white bass transferred approximately the same proportion of chlordanes and other organochlorine contaminants as lipids to their eggs (*2*). Second generation guppies, born in the period of elimination (i.e., after transfer to clean water), contained slightly less PCB than their mothers (*7*). However, because egg content was not measured directly in that study, it is uncertain whether the lipids transferred to the eggs were also lower in PCB content than other tissues of the mother fish or whether the dilution occurred after hatching into clean water.

Generally, contaminant concentrations in lipid compartments within an organism are considered to be equivalent for modeling purposes. We have determined that this is not the case for mayflies and their eggs. Two processes which may affect the distribution of nonpolar contaminants within lipid tissues of an organism are as follows: (a) nonequilibrium conditions, i.e., insufficient time to complete transport between lipid compartments; (b) lipid composition, with polar lipids containing lower concentrations than nonpolar lipids. If production mechanisms were different for lipids in the fat body used to produce the egg mass versus those in other tissues of the mayfly, one would expect differences in compositional makeup such as lower levels of metabolites in one. The chlordane signatures differed only slightly (Figure 1), both dominated by tNONA with metabolites comprising the remainder. Eggs contained more HPX; however, those results were so variable that it was difficult to compare that portion of the signature, and we cannot rule out a comparable proportion in adults.

One possible mechanism for the inequality between chlordane content in the lipids of eggs and adult mayflies is that the lipid tissues are not in equilibrium. Equilibrium is reached when the system, both within the organism and its environs, is at steady state and sufficient time has passed such that transfer mechanisms have had time to complete distribution. The time required for equilibrium to be reached for PCBs in fish was determined to be greater than 1 year (*27*). Obviously for a mayfly with a lifespan of about 1 month, equilibrium might not be obtained. Precisely what contact the egg mass lipid has with other lipid tissues or when it is produced is unclear. However, the mayfly grows exponentially, acquiring ≥90% of its total biomass in the last 7–10 d of its aquatic life stage at 20 °C (*11*). Thus, most tissue is less than 1 week old.

Growth is generally believed to dilute organochlorine content in fats (*28, 29*). If lipids are acquired for eggs over the same period as for other lipid tissues, then they should be diluted equally. If they were acquired during the growth spurt at the end, they should be more dilute than other lipid tissues of the mayfly. That is not the case. They would have to be formed early and stored prior to production of other lipid stores for this mechanism to explain the results. We noted previously that the chlordane-exposed mayflies were slower to emerge and accumulated more lipid in non-egg tissues than controls. This extra growth would account for a dilution of chlordane up to 30% in adult tissues if egg generation was not similarly delayed. On the other hand, because the mayfly ceases eating for the 24-h period after emergence and prior to ovipositing, her non-egg lipid tissues would be used for energy, and thus those fat stores should be more concentrated in contaminants because lipids are more readily metabolized than contaminants such as chlordane (*29*).

Again, we know this was not the case. Excretion of the contaminants would have to exceed use of the lipids for fuel. However, there is no evidence suggesting that mayflies continue to excrete after emergence.

One concept in recent modeling efforts is the use of pharmacological or compartment modeling, where organisms are considered as a set of compartments rather than as a whole (*30*). Compartments within the organisms would be characterized by rate constants, fugacity differentials (i.e., concentration gradients), or clearance constants based on high versus low perfusion tissues. Barron and co-workers (*30*) state that uptake and depuration are dependent on the "blood perfusion rate" and efficiency by which organs extract the chemical from the circulating fluid. For mammals, they stated that the blood flow to adipose tissues was only a few percent of that which flows to organs such as the brain and heart. After egg generation or encapsulation, eggs would no longer be exposed to a high perfusion rate. Because they were formed from a fat body (*31*), they probably could have been classified as a low perfusion compartment even prior to encapsulation. Other tissues of the mayfly would be in contact with circulating fluids and as such would be high perfusion compartments. High perfusion rates might explain a dilution of contaminants in tissues after emergence when feeding and, thus, uptake are halted. However, this is counterintuitive when considering the general rule of thumb that starvation generally leads to an increase in relative concentration of nonpolar pollutants due to the relatively higher loss of lipids (*29*). Also, as mentioned above, it is unlikely that excretion continues in mayfly adults.

Differences in lipid composition among various mayfly tissues may be the most likely explanation of our results because it affects the capacity of lipids for binding nonpolar contaminants. Lipids range in polarity, with the least polar having the highest capacity for solubilizing nonpolar pollutants. Research on lipids in fish eggs and juveniles showed a tendency for the content of the pesticide lindane to correlate with type of lipid, i.e., polar lipids contained less than neutral lipids (*6*). The fish eggs had more polar lipid content, converting toward more nonpolar lipids as they developed. If mayfly eggs also contain a relatively higher proportion of polar lipids than other adult tissues, then they should carry lower contaminant loadings per milligram lipid. Our results, however, suggest the opposite. Bierman (*32*, and references cited therein) noted a seasonal variation for lipid-normalized bioconcentration factors for some benthic organisms, suggesting that differences in the quality and composition of lipids may be a factor in the variance. A hypothesis for our mayfly is that the fat body consists of a proportionally higher concentration of nonpolar lipids than other tissues. Work on the nature of lipid compartments in mayflies is necessary to determine whether this hypothesis can explain the disequilibrium in chlordane content between the adults and their eggs.

Summary

Mayfly eggs produced by mothers who were exposed to sublethal levels of technical chlordane were found to contain several times the concentration of chlordane compounds as other tissues within the adult mayfly. Because of the magnitude of the growth of mayflies over their month-long aquatic lifespan, the impact of the higher contaminant load would primarily affect early development or survivorship of larvae. Chlordane concentrations delivered from the mother would be diluted by several orders of magnitude during the subsequent larval growth period. We found no obvious ill effects to the second generation of exposed individuals; however, we did not measure susceptibility to further toxic exposure, nor did we monitor subsequent generations. In addition to impact on second generation mayfly larvae, the disequilibrium would affect the exposure of predators to both the adults and the eggs. Fish feeding on mature larvae or newly emerged adults would be exposed to 70% more chlordane than those feeding on adults after oviposition. Should meiofauna, which typically feed on diatoms, feed instead on these eggs (1100 ppb), they would be exposed to a dose of chlordane 100 times the concentration of that in diatoms (algae exposed to 10.7 μg/L of chlordane contained 26 ppb; thus, by correlary, algae exposed to 4.5 μg/L would contain $\sim$10 ppb).

The factors driving the disequilibrium between the adult mayfly tissue and their eggs may include that the slower growth of chlordane-exposed adults results in a growth of adult lipid tissues (up to 30% dilution); that lipids in eggs were less accessible, i.e., in low perfusion regions; and/or that lipid content varied and thus differed in capacity for the accumulation of chlordanes. That the chlordane signature in the eggs was roughly the same as that in other adult tissues indicated that processes involved in the mayfly's transformation of chlordane acted prior to those involved in the sequestering of lipids into different compartments. While lipids in the egg mass may be less "exposed" to loss processes, ultimately they were not less susceptible to metabolism. This provides an interesting puzzle, suggesting a need for further investigation of the processes involved in intraorganism contaminant transport. It also emphasizes the danger of using a single lipid-based value for modeling contaminants in biota. Because egg hatch success is more sensitive to ovarian or egg content of contaminants (*33*), impact of contaminants on offspring would be best correlated to concentrations in ovaries or eggs.

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