Assessment of Human Health Risks from Chemically Contaminated Lake Fishes In Greece

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ABSTRACT

Objectives were to conduct screening level surveys of locally consumed fish tissues in vicinities of two lakes (Kastoria and Pamvotis) in Greece to determine the presence of halogenated organic compounds and determine carcinogenic and non-carcinogenic human health risks associated with the consumption of sampled fish tissues. Results estimate the Incremental Lifetime Cancer Risks (ILCR) and Hazard Index (HI) values for the two local populations using site-specific population data. These results were compared to analyses conducted using U.S. Environmental Protection Agency default values in an effort to determine the applicability of USEPA default values to assessments of risks in non-U.S. populations. Using site specific data, 87% of the mean ILCRs calculated for total populations and sub-populations (i.e. female adult, female youth, male adult and male youth) consuming fishes from the two lakes we studied were above USEPA’s acceptable cancer risk of 1.0×10^{-6}; 53% of the mean HIs were greater than 1.0. The USEPA default value (0.054 kg/d) for ingestion rate (IR) is considerably lower than the mean site specific IRs derived from populations in vicinity of Lake Kastoria (0.20; min. = 0.09; max. = 0.29 kg/d) and Lake Pamvotis (0.10; min. = 0.01; max. = 0.21 kg/d). These differences point to the need for the development of default values specific to the regions and population consumption patterns within Greece.

Keywords: human health risk assessment, PCB, pesticides, Greece, European Union
The hazards of pesticides are receiving increased attention in European Union (EU) countries. For example, the European Environmental Agency (EEA) of the EU removed a large number of pesticides from the market in July 2003 (Pesticide News, 2003). Pesticide use in Greece during 1989 was 7,811 tons, and has likely increased significantly since that time due to changing dynamics in Greece's agribusiness (Dassenakis, 2000; Lekakis, 1998).

Published studies of human health risks associated with pesticide exposure in Greece are limited. Dolasakis et al. (2001) have reported on occupational exposure to pesticides currently used in Crete, finding that female greenhouse workers had elevated incidences of mammogram abnormalities, including tumors. Additionally, Schinas et al. (2000) have reported that organochlorine pesticide residues in human breast milk from southwest Greece are correlated with dietary intake of pesticides.

Some investigators in Greece have explored the presence of halogenated organic compounds and determine carcinogenic and non-carcinogenic human health risks associated with the consumption of fish tissues. Salaris and coworkers (2004) studied organochlorine levels in muscle of Lepidopsethobius boesmi (four-spotted megrim, an edible flatfish) in the Aegean Sea and found concentrations of DDT ranging from 12.5-32.3 ng/g, and those of PCBs between 4.5-12.1 ng/g. Charizopoulos and Papadopoulos-Mourkidiou (1999) found one or more pesticides in 90% of 205 rainwater samples. Atrazine was measurable in 30% of the 205 samples. In Greece's freshwater Lake Kastoria, all four pesticides sampled for were found at all locations sampled. Concentrations of atrazine (avg. =0.7 mg/l; range =0.5-0.9 mg/l) land endosulfan (avg. =0.51 mg/l; range =0.2-1.0 mg/l) were higher than those of fenathion (avg. =0.053 mg/l), chromate (avg. =0.056 mg/l) (Bobori, 1998).

However, no comprehensive data base of contamination in groundwater, surface water, or dietary commodities could be located, suggesting that these potential contaminant inputs are not routinely monitored for risk management purposes. Additionally, a thorough review of the literature indicates no formal data base of contamination in groundwater, surface water, or dietary commodities could be located, suggesting that these potential contaminant inputs are not routinely monitored for risk management purposes.

In the Agricultural University of Athens, 2004), a total of 27 samples were analyzed for polychlorinated Biphenyls (PCBs) by congener analysis and organochlorine pesticide burdens. Samples were homogenized and lyophilized, then sub-sampled and subjected to enhanced solvent extraction (Schantz, 1997) using methylene chloride as the solvent. Multiple surrogate standards (PCBs: PCB-30, PCB-65, PCB-121 and PCB-204) were added prior to sample extraction to span the molecular weight range of the targeted analytes. Size exclusion chromatography (SEC) purification of extracts was accomplished using an HPLC column using methylene chloride as the solvent at a flow rate of 5 ml/min. Polarity separation of post-SEC extracts was conducted on 2000 mg silica gel solid phase extraction (SPE) columns. Extracts were separated for use in fish advisories.
processed. The second silica gel fraction (eluted with 6 ml of 60:40 hexane/methylene chloride) contained the PCBs and organochlorine pesticides. Solvent volumes were reduced under purified nitrogen.

The second SPE fractions were then spiked with an internal standard, pentachlorobenzene, for quantitation purposes. Separation and detection of contaminants were accomplished by high-resolution gas chromatography (GC) with electrolytic conductivity detection (ELCD), operated in the halogener selective mode. The ELCD selectively responds to halogenated analytes, i.e. those containing Cl, Br, F or I. A 1 to 2 ul injection of each purified extract was made onto a 60 m DB-5 column (0.32 mm ID and 0.25 um film thickness), using splitless injection and helium carrier gas. Organochlorine compound identifications were made via halogen retention indices (HRIs) and an existing VIMS analyte library. Extracts derived from VIMS quality control samples #001 and #005 were also subjected to full scan GC/MS to confirm compound identities indicated by the retention indices. Data were corrected for recovery of the PCB-204 surrogate.

Quality control measures also included the coincident processing of three blanks, consisting of pre-ignited sodium sulfate, with each set of samples lohexiphalized. Each sample was spiked with the fourth PCB surrogate standards to determine analyte recovery rates. A certified reference material (CRM) fish (Carp-1, US National Institute of Standards and Technology (NIST)), recently analyzed for PCBs and selected pesticides to ascertain accuracy of the VIMS methodology (Schantz, 1997) was also analyzed with the Greek samples, as were four samples of C. carpio from the New River, Virginia, USA, which were also recently analyzed by VIMS.

Risk Analysis: Analyses of health risks associated with consumption of fish containing the identified compounds were performed using US Environmental Protection Agency (USEPA) Risk Assessment Guidance for Superfund (RAGS) (USEPA 1989; 1991a). In general, analyses for determining carcinogenic health risks: 1. determine the Intake factor (IF) term by inputing either site specific or default population exposure information; 2. determine an Exposure Point Concentration (EPC) for an identified compound; 3. determine a Chronic Daily Intake (CDI) value for each compound by multiplying the EPC by the IF; and 4. determine an Incremental Lifetime Cancer Risk (ILCR) by applying a compound’s cancer potency factor (CSF) from EPA’s Integrated Risk Information System (IRIS, 2002) to the CDI. Individual ILCRs are then analyzed to determine the range of risk as well as the mean risk for the population.

Analyses for determining non-carcinogenic health effects follow much the same general procedure but use an Oral Reference Dose (RID) in place of the CSF and generate a final value termed a Hazard Index (HI). Individual HIS are analyzed to determine the range of non-carcinogenic risk as well as a mean non-carcinogenic risk. Compounds were included in the risk analyses if there was sufficient toxicological data in IRIS to support the algorithms or if they represented breakdown products of parent compounds that could be readily characterized in IRIS. Breakdown products for chlordane, DDT, DDE and DDD were grouped with their parent compounds.

The algorithm for calculating the IF was:

\[ IF = IR \times FI \times ED \times EF \times BW \times AT \]

Where:
- **IR** = ingestion rate (kg/d or kg/meal)
- **FI** = fraction ingested from contaminated source
- **ED** = exposure duration (yrs)
- **EF** = exposure frequency (d/yr or meals/yr)
- **BW** = body weight (kg)
- **AT** = averaging time (d)

Determinations of IF used both site-specific exposure data from Mauvais and Grimes (2005), and RAGS default exposure inputs for IR, ED, EF and BW in USEPA (1991a) to determine the applicability of USEPA default values to assessments of human health risks in populations outside the United States. The value of FI was set at 1.0 as data from Mauvais and Grimes (2005) indicated local populations obtained their fish primarily from the respective lake sampled in the region. Values for AT were set at 70 years for analyses of carcinogenic risks and 30 years for non-carcinogenic risks (USEPA 1991a). Default values used were: IR= 0.054 kg/d, FI= 1, AT-cancer= 25,550 d (70 yr x 365 d/yr), AT-non-cancer= 10,950 d (30 yr x 365 d/yr), ED= 30 years, EF= 350 d/yr, and BW= 70 kg -adult, (IRIS, 2002; USEPA 1991a). Due to the relatively small size of most of the data sets, i.e. < six samples, many EPCs were set at the maximum detected compound concentrations. Data sets with greater than six samples used the 95% upper confidence limit (UCL) of the compound concentrations as the EPC (EPA 1989).

The algorithm for calculating chronic daily intake (CDI) of a target compound was:

\[ CDI = \text{mg/kg-d} = \text{EPC} \times \text{IF} \]

Where:
- **EPC** = concentration of identified compound in tissue (mg/kg)
- **IF** = Intake factor

The CDI for each compound was multiplied by the CSF to calculate the ILCR estimate for that compound. Estimated ILCRs were summed for all compounds identified in a species collected from the respective lakes to determine an individual’s total ILCR estimate for exposure to all target compounds identified in the species. Individual total ILCRs were analyzed to determine minimum, maximum and mean risk estimates for the general fish consuming population, and four sub-populations: adult men, adult women, male children (age < 18 years) and female children (age < 18 years). Tests for significant differences in sub-population ILCR estimates were made using the SAS GLM procedure followed by Duncan’s Multiple Range Test (SAS, 2002). Estimated HIS were summed for all compounds identified in a species collected from the respective lakes to determine an individual’s HI estimate for exposure to all target compounds identified in the species. Individual HIS were analyzed to determine minimum, maximum and mean HI estimates for the general fish consuming population, and the adult men, adult women, male children (age<18 years) and female children (age<18 years) sub-populations. Tests for significant differences in sub-population HI estimates were made using the SAS GLM procedure followed by Duncan’s Multiple Range Test (SAS, 2002). The RID for Acorus 1254 was used to assess total risks for total PCBs as this PCB mixture is reflective of the PCB congeners that bioaccumulate in fish (IRIS 2002).
Calculation of ILCRs and HI values for S. aristotelis from Lake Pamvotis were made separately from the calculations for C. carpio and A. anguilla because surveys for consumption of S. aristotelis were conducted on different dates and thus sampled a different population than that surveyed for consumption habits of C. carpio and A. anguilla.

RESULTS

Fish Collection and Processing: A total of 27 fish from two species groups (P. fluviatilis, 17 and R. rutilus, 10) were collected from Lake Kastoria, and a total of 37 fish from three species (C. carpio, 7; A. anguilla, 7, and S. aristotelis, 23) were collected from Lake Pamvotis. Lake Kastoria collections yielded sufficient amounts of tissue samples for four fillet samples of the predator P. fluviatilis and two fillet samples of the prey species R. rutilus. Lake Pamvotis collections resulted in five fillet samples and five roe samples from the bottom feeder C. carpio, seven fillet samples from the bottom feeder A. anguilla, and four fillet samples from the predator S. aristotelis. Percent difference in total length of samples composited for analysis ranged from 1.9 to 16.7 percent, well within USEPA recommended ≤ 25 percent range (USEPA 1993).

Sample Analysis:

Quality control measures: Contamination detectable in the sodium sulfate blanks was typically less than 1 ug/kg (dry weight basis) per component. Recovery of PCBs in surrogate samples ranged from 110 to 133%. These recovery values were used to correct results from the extraction process as the majority of chlorinated analytes eluted between PCB-121 and PCB-204 and had intermediate volatilities, leaning towards that of PCB-204.

Total PCB values were in excellent agreement with the total PCB values for the 25 peaks quantitated by NIST for the CRM fish. Values for 4,4-DDT and 4,4-DDE for the CRM fish agreement with NIST values. Recovery of these samples were also in excellent agreement with the NIST values for the CRM fish. Recovery of PCBs (congeners 153/132, 138 and 180). Other halogenated organics identified in Lake Kastoria samples ranged from 0.1-2.7 ppb (Table 1).

TABLE 1. Exposure point concentrations (EPC) by chemical and species (Perca fluviatilis, Rutillus rutilus, Anguilla anguilla, Cyprinus carpio, and Silurus aristotelis) used in analyses of human health risks for populations living in vicinities of Lake Kastoria and Lake Pamvotis, Greece; and concentration ranges for unidentified halogenated organic compounds found in tissue samples.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Lake Kastoria P. fluviatilis</th>
<th>Lake Pamvotis A. anguilla</th>
<th>25% range</th>
</tr>
</thead>
<tbody>
<tr>
<td>alpha-BHC</td>
<td>0.04</td>
<td>0.04</td>
<td>0.49</td>
</tr>
<tr>
<td>beta-BHC</td>
<td>0.05</td>
<td>0.05</td>
<td>0.33</td>
</tr>
<tr>
<td>gamma-BHC</td>
<td>0.05</td>
<td>0.05</td>
<td>0.33</td>
</tr>
<tr>
<td>hexachlorobenzene</td>
<td>0.03</td>
<td>0.03</td>
<td>0.24</td>
</tr>
<tr>
<td>heptachlorophenoxyde</td>
<td>0.04</td>
<td>0.04</td>
<td>0.33</td>
</tr>
<tr>
<td>chlordane</td>
<td>0.18</td>
<td>0.18</td>
<td>0.33</td>
</tr>
<tr>
<td>DDE</td>
<td>0.15</td>
<td>0.15</td>
<td>0.33</td>
</tr>
<tr>
<td>DDD</td>
<td>0.23</td>
<td>0.23</td>
<td>0.33</td>
</tr>
<tr>
<td>unidentified (range)</td>
<td>0.1-0.5</td>
<td>0.1-0.5</td>
<td>0.1-0.5</td>
</tr>
</tbody>
</table>

Unidentified halogenated organic compounds were not quantified in C. carpio egg samples.

The risk analyses were: Total PCB (includes all identified PCB congeners); Alpha Benzene Hexa Chlorine (BHC); Beta BHC; Gamma BHC; Hexachlorobenzene; Chlorodane (includes 2,3,7,8-TCDD and 2,3,7,8-TCDF); and DDE (includes 4,4-DDE and 4,4-DDD). Exposure Point Concentrations were selected from maximum detected compound concentrations in all species except A. anguilla, which had a sufficiently robust data set to use the 95% UCL as the EPC for most compounds (Table 1).

Determination of UF:

Not all individuals surveyed in Maurakis et al. (2005) consumed all species of fish collected from a particular lake. No UF values were calculated for 11 individuals surveyed from the Lake Kastoria population as they did not consume either P. fluviatilis or R. rutilus. Therefore, these individuals were not included in estimations of risks using site specific population data. Five individuals reported consuming P. fluviatilis but not R. rutilus, while two individuals reported consuming R. rutilus but not P. fluviatilis. As a result of these differing consumption patterns, UF values were calculated for 29 individuals that consumed both species and therefore these data combinations resulted in the calculation of 34 UF values for the Lake Kastoria risk assessment.

Not all site were calculated for 16 individuals surveyed from the Lake Pamvotis population as they did not consume either C. carpio or A. anguilla, and therefore these individuals were not included in estimations of risks using site specific population data. Fifteen individuals reported consuming C. carpio but not A. anguilla, and four individuals reported consuming A. anguilla but not C. carpio. A. anguilla was significantly resistant to environmental degradation and their concentrations would not be expected to be significantly altered by the levels of decomposition associated with the samples.
consumption patterns, IF values were calculated for 27 and 15 individuals that consumed C. carpio and A. anguilla, respectively. These data combinations resulted in the calculation of 30 IF values for the Lake Pamvotis risk assessment for these two species. No IF values were calculated for 27 individuals surveyed from the Lake Pamvotis population as they did not consume S. aristotelis; therefore these individuals were not included in estimations of risks using site-specific population data. Deletion of these 27 individuals resulted in the calculation of 16 IF values used to calculate risk for consumption of S. aristotelis.

**USEPA Default IF:**
The USEPA default IF value was calculated from the default inputs presented above. Determinations of ILCRs and HIs using the default IF do not consider the effects of site specific age, sex, body weight or other exposure factor variability.

**Determination of ILCR and HI:**

**Lake Kastoria - P. fluviatilis and R. rutilus consuming population:**
The mean site specific ILCR for the general population was 3.80E-5 (range=3.1E-7 - 2.0E-4). The ILCR from the USEPA default model was 1.7E-5. Mean site specific ILCRs in the sub-populations ranged from 1.3E-5 to 6.9E-5 in female children. The mean ILCR in adult men was significantly greater than mean ILCRs in other Lake Kastoria sub-populations (Table 2).

The mean site specific HI for the general population was 1.2 (range=0.1-4.2) compared to 0.32 derived from the USEPA default model. Mean HI values in the sub-populations ranged from 0.9 in adult women and female children to 1.9 in male children. Mean HIs were not significantly different between sub-populations (Table 2).

**Lake Pamvotis - C. carpio and A. anguilla consuming population:**
The mean site specific ILCR for the general population was 1.20E-4 (range=2.2E-3 - 4.5E-4). The ILCR from the USEPA default model was 9.31E-5. Mean site specific ILCRs in the sub-populations ranged from 1.0E-5 in female children to 2.3E-4 in male adults. The mean ILCR in adult men was significantly greater than mean ILCRs in other Lake Pamvotis sub-populations (Table 2).

The mean site specific HI in the general population was 2.9 (range=0.1-9.3) compared to 0.34 derived from the USEPA default model. Mean HI values in the sub-populations ranged from 1.1 in female children to 5.0 in adult men. The mean HI in male adults was significantly greater than the mean HIs in other Lake Pamvotis sub-populations (Table 2).

**Lake Pamvotis - S. aristotelis consuming population:**
The mean site specific ILCR for the general population was 1.8E-5 (range=6.4E-7 - 1.1E-4). The ILCR from the USEPA default model was 9.3E-5. Mean site specific ILCRs in the sub-populations ranged from 6.2E-5 to 3.0E-5 in adult women. Mean ILCRs were not significantly different between sub-populations (Table 2).

The mean site specific HI for the general population was 0.5 (range=0.0-2.3) compared to 0.34 derived from the USEPA default model. Mean HI values in the sub-populations ranged from 0.3 in adult men to 0.7 in adult women and female children. Mean HIs were not significantly different between sub-populations (Table 2).

**DISCUSSION**

Our analyses are based on guidelines, risk models, and default values of the USEPA and not those of the EEA as the latter has not published human health risk models and default values for contaminants in fish products. However, in 2003 the European...
Commission did list cancer risks from environmental, diet, and genetic factors as a priority (EEA, 2004). USEPA uses an ILCR risk range of 1E-10 to 1E-05, and an HI <1 (USEPA 1989, USEPA 1991a). The guidance is widely interpreted as setting an ILCR of 1E-06 as the rate of occurrence of cancer that could be expected in the absence of xeno-biotic compounds (i.e. the point of departure). Increasing departures from this level of risk suggests an increasing need for actions to manage risks to human health, as would HIS elevated to 1.0 or greater. Once ILCRs approach or exceed 1E-04, and HIS approach or exceed 1.0, EPA will often require remedial action to be taken to reduce risk factors to levels conducive to management through institutional or other controls.

Using site specific data, 87% of the mean ILCRs calculated for total populations and sub-populations (i.e. female adult, female youth, male adult and male youth) consuming fishes from the two lakes we studied were above USEPA's acceptable cancer risk for the mean site specific IRs derived from populations in vicinity of Lake Kastoria population at greatest risk. For S. a. aristotelis, and HI values (2.9) for the general population consuming A. anguilla and C. carpio from Lake Pamvotis exceed the levels where the USEPA would often require remedial action. The sub-population estimated to be at the greatest risk for carcinogenic health risks is adult males (mean ILCR=2.4E-04, mean HI=5.0).

In Lake Kastoria, mean ILCR (3.8E-05, max.=2.0E-04) for the general population consuming P. fluviatilis and R. rutilus also exceed USEPA's point of departure for cancer risk but is below the level where USEPA would likely require remedial action beyond some form of institutional control such as risk communication and / or a consumption advisory. However, the mean HI value for the general population (1.2, max.=9.3) is above this level where the USEPA would likely require remedial action beyond the use of institutional controls. As the site specific mean ILCR (2.0E-05) and HI values (2.9) for the general population consuming A. anguilla and C. carpio from Lake Pamvotis exceed the levels where the USEPA would often require remedial action the sub-population estimated to be at the greatest risk for carcinogenic health risks is adult males (mean ILCR=2.4E-04, mean HI=5.0).

HEALTH RISKS FROM FISH IN GREECE

In both lakes, primary drivers were lipophilic compounds and not risk contributions from non-lipophilic compounds, unidentifiable halogenated organics or metals. Accordingly, the rivers, lakes, seas sediments in and around Greece should be sampled and analyzed for organic and inorganic contaminants and the results inventoried in a GIS based data warehouse to guide and focus future risk assessment efforts.

Risk management decisions, such as recommending consumption limits, are beyond the scope of this study and will require a fuller characterization of both exposure and consumption issues if cost effective actions are to be correctly identified. If consumption patterns in other southern European Union countries are comparable to those of Greece, the EU should consider conducting human biomonitoring projects. The need for such studies will continue to grow as the issue becomes more evident in
the light of globalization and the associated growth of international environmentalism such as that reflected in the compliance provisions of HACCP (USFDA, 2001), Codex Maximum Residue Limits for Pesticides and Extraneous Maximum Residue Limits (Codex Alimentarius Commission, 1997) and guidelines of World Health Organization (WHO, 1999).

ACKNOWLEDGEMENTS

Funded in part by the Thomas F. and Kate Miller Jeffress Memorial Trust, Science Museum of Virginia, Aristotle University, and University of Richmond. E. G. Maurakis, D. V. Grimes, and D. Bobori made collections, analyzed data, and prepared the manuscript; R. Hale of Virginia Institute of Marine Science performed qualitative and quantitative analyses of fish tissue samples; and J. Jones, VA Dept. of Environmental Quality, calculated risk indices.

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