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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 $1.0E^{-06}$; 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

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INTRODUCTION

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 scant. Dolapsakis et al. (2001) have reported on occupational exposure to pesticides currently used in Crete, finding that female greenhouse workers had elevated incidencies 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 also reported on the concentrations of pesticides in water and fish. For example, Georgakopoulos-Gregoriades and Vassilopoupou (2004) studied organochlorine levels in muscle of *Lepidorhombus boscii* (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 Papadopoulou-Mourkidou (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)and endosulfan (avg.=0.51 mg/l; range=0.2-1.0 mg/l) were higher than those of fenathion (avg.=0.053 mg/l), chronomeb (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 human health risk assessments of pesticides in fish tissues in Greece have been published (pers. comm. Joachim Scholderer, MAPP Centre at the Aarhus School of Business, Denmark, 2004; Joyce Tait, Univ. Edinburgh, 2004; George Chryssochoidis, Agribusiness Laboratory, Agricultural University of Athens, 2004).

Despite this apparent lack of monitoring and assessment of the fate and transport of pesticides and other contaminants in the environment, the Greek government has recently authorized the use of nine organic pesticides with known human health impact potentials: endosulfan, anthracene, simazine, trifluralin, alachlor, atrazine, chlorpyrifos, diuron, isoproturon, chlorfenvinphos, and naphthalene (EEA, 2000).

Our 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.

METHODS AND MATERIALS

Fish Collection and Processing: Species collected were those consumed by local populations (Maurakis et al., 2005). Lake Kastoria fish were obtained from local fishermen immediately after they finished fishing their gill net (58 mm bar mesh) on 19 June 2002. For Lake Pamvotis, live fish maintained in lake water pumped once through tanks, were purchased from a fish tavern on the Island of Ioannina on 21 June 2002.

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Collections were placed in plastic bags, labeled with collection site, species, and date of collection, placed on ice and transported to University of Ioannina within 24 hours where they were stored at –20 C until processed.

Sample processing followed the *Guidance For Assessing Chemical Contaminant Data For Use In Fish Advisories* (USEPA 1993) *Guidance for Conducting Fish and Wildlife Consumption Surveys* (USEPA 1998) and the good laboratory practices of EU Directive 93/99EEC outlined in Hill (1999). Stainless steel equipment was used in processing samples for organics analysis. Prior to preparing each sample, equipment was washed with a detergent solution, rinsed with tap water, wiped with isopropanol and rinsed with deionized water. Work surfaces were wiped down with isopropanol, allowed to dry and covered with heavy-duty aluminum foil, which was changed after each sample was processed.

Total length (mm) and weight (g) were recorded for each specimen to determine sample variability within species. Species processed as whole body samples (*Rutilus rutilus*) were scaled, gutted and rinsed with deionized water. Species processed as skin-on fillets (*Perca fluviatilis*) were scaled prior to filleting (belly flap discarded) and rinsed with deionized water. Species processed as skin-off fillets (*Cyprinus carpio*, *Anguilla anguilla*, *Silurius arestotilis*) were partially scaled as needed to facilitate filleting and then skinned after filleting. Carp roe was resected for sampling as consumption survey data (Maurakis et al., 2005) indicated carp roe was also consumed. Tissue samples were weighed on a digital scale covered with heavy-duty aluminum foil which was replaced after each weighing. If fillets from an individual fish had a total weight of less than 200 grams, fillets were composited with one or more fillets from the same species to generate a tissue sample of at least 200 grams (USEPA 1993). Fillet samples were wrapped in heavy duty aluminum foil with a sample identification tag labeled for: sample location, species, sample type (individual or composite), total length(s) and weight, field sample number, and sample date. Carp roe samples were placed in plastic jars with Teflon lined lids.

Sample Analysis: All samples were stored at –20 C until they were packed in ice and shipped by overnight courier from Thesoloniki, Greece to Richmond, Virginia. In Richmond, samples were stored at –5 C until they were packed in ice and transported to the Department of Environmental and Aquatic Animal Health at the Virginia Institute of Marine Science (VIMS), College of William and Mary, Gloucester Point, Virginia, USA. Final sample preparation and chemical analyses were conducted using VIMS Analytical Protocol for Hazardous Organic Chemicals in Environmental Samples (Hale, 1994) with modest modifications to improve extraction results and sample throughput.

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 into two silica derived fractions. The first (eluted with 5 ml of 100% hexane) contained aliphatics and was not further

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 halogen 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 lyophilized. Each sample was also spiked with the four 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 effect risks follow much the same general procedure but use an Oral Reference Dose (RfD) 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:

$$IF = IR*FI*ED*EF/(BW*AT)$$

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 Maurakis 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 Maurakis 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:

$$CDI\ (mg/kg\text{-}d) = EPC*IF$$

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 RfD for Aroclor 1254 was used to assess non-cancer risks for total PCBs as this PCB mixture is reflective of the PCB congeners that bioaccumulate in fish (IRIS 2002).

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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 Pamovits 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 $\leq$ 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 PCB-204 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 were also in excellent agreement with the NIST values. Results for 4,4-DDD were low, about 45% of expected. As this compound was consistently found in all samples, the low recoveries may underestimate the concentrations of this compound in fish tissues. The percent differences between PCB extraction results and those of the earlier extraction results for the New River fish were 2-4%.

Samples showed some signs of decomposition upon receipt by the VIMS laboratory due to thawing during transport. Although lipid composition was likely altered by sample decomposition, the halogenated analytes targeted for analysis are generally resistant to environmental degradation and their concentrations would not be expected to be significantly altered by the levels of decomposition associated with the samples. This assumption is supported by the pattern of increasing halogenated organics concentrations with increasing percent lipid in the samples.

Sample results: The dominant halogenated organics detected within parameters of the analytical protocol were degradation products of DDT, principally 4,4'-DDE, and PCBs (congeners 153/132, 138 and 180). Other halogenated organics identified in most samples included: hexachlorobenzene and associated degradation products; Chlordane and associated degradation products; and several un-identified halogenated organics. Concentrations of the 16 un-identified halogenated organics in Lake Pamvotis samples ranged from 0.1–18.1 ppb. Concentrations of the 19 un-identified halogenated organics in Lake Kastoria samples ranged from 0.1–2.7 ppb (Table 1).

Risk Analysis:

Descriptive statistics and resultant EPC values: The ten compound groups used in the risk analyses were: Total PCB (includes all identified PCB congeners); Alpha Benzene Hexa Chlorine (BHC); Beta BHC; Gamma BHC; Hexachlorobenzene; Chlordane (includes cis-chlordane, trans-chlordane, and chlordane); DDE (includes 2,4 DDE and 4,4 DDE); DDT (includes DDT, 4,4 DDT, and DDT related); DDD (includes 4,4 DDD, and DDD-olefin); and Heptachlor epoxide. 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).

TABLE 1. Exposure point concentrations (EPC) by chemical and species (*Perca fluviatilis*, *Rutilus rutilus*, *Anguilla anguilla*, *Cyprinus carpio*, and *Silurus aritotelis*) 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.

	Lake Kastoria		Lake Pamvotis			
(ppb)	*P. fluviatilis*[1]	*R. rutilus*[1]	*A. anguilla*[1,2]	*C. carpio*[1]	*C. carpio* eggs[1]	*S. aristotelis*[1]
Chemical						
alpha-BHC		0.04	0.23 [2]			
beta-BHC			0.05 [1]			
gamma-BHC		0.05	0.64 [2]	0.05		
hexachlorobenzene	0.03	0.13	0.78 [2]	0.15	0.19	0.47
heptachlor epoxide		0.04	0.41 [2]	0.11		0.28
chlordane	0.18	1.17	2.53 [2]	1.87	1.15	3.65
DDD	0.15	1.83	10.34 [2]	3.38	2.21	12.16
DDE	4.7	45	94.5 [2]	34.54	23.61	86.97
DDT	0.08	2.34	6.85 [2]	2.2	1.11	3.47
Total PCBs	1.61	7.0	36.8 [2]	13.7	10.4	30.9
Unidentified (range)[3]	0.1-0.5	0.1-2.7	0.1-4.4	0.2-18.1	- [4]	0.1-5.7

[1]=maximum concentration used as EPC due to sample size <6.
[2]=95% UCL used as EPC when sample size >6.
[3]=No risk assessments were performed for unidentified halogenated organic compounds.
[4]=Unidentified halogenated organic compounds were not quantified in *C. carpio* egg samples.

Determination of IF:

Not all individuals surveyed in Maurakis et al. (2005) consumed all species of fish collected from a particular lake. No IF 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, IF values were calculated for 32 and 29 individuals that consumed *P. fluviatilis* and *R. rutilus*, respectively. These data combinations resulted in the calculation of 34 IF values for the Lake Kastoria risk assessment.

No IF values 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*, three individuals reported consuming *A. anguilla* but not *C. carpio*. As a result of these differing

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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=$3.10E^{-7}$ – $2.00E^{-4}$). The ILCR from the USEPA default model was $1.17E^{-5}$. Mean site specific ILCRs in the sub-populations ranged from $1.3E^{-5}$ in female children to $6.9E^{-5}$ in male adults. 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=$2.2E^{-7}$–$4.5E^{-4}$). 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 $2.4E^{-4}$ 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 3.49 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. arestotelis consuming population:

The mean site specific ILCR for the general population was $1.8E^{-5}$ (range=$6.4E^{-7}$ – $1.1\ E^{-4}$). The ILCR from the USEPA default model was $9.3E^{-5}$. Mean site specific ILCRs in the sub-populations ranged from $6.2E^{-6}$ in male children 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 3.49 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

TABLE 2. Site specific mean, minimum, and maximum Incremental Lifetime Cancer Risks (ILCR) and Hazard Indexs (HI) for the general population and subpopulations (adult male, adult female, male youth (age<18 years), and female youth (age<18 years) consuming *Percafluviatilis* and *Rutilus rutilus* in Lake Kastoria, and *Anguilla anguilla*, *Cyprinus carpio*, and *Silurus aristotelis* in Lake Pamvotis, Ioannina, Greece in 2002.

	Lake Kastoria			Pamvotis (*A. anguilla* & *C. carpio*)			Pamvotis (*S. aristotelis*)		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Total population									
ILCR:	3.10E-07	2.00E-04	3.80E-05	2.20E-07	4.50E-04	1.20E-04	6.40E-07	1.10E-04	1.80E-05
HI:	0.1	4.2	1.2	0.1	9.3	2.9	0.0	2.3	0.5
Adult men									
ILCR	6.90E-06	2.00E-04	6.9E-05 (*)	3.70E-05	4.50E-04	2.4E-04 (*)	6.40E-07	5.30E-05	1.40E-05
HI	0.1	3.9	1.3	0.9	9.3	5 (*)	0.0	0.8	0.3
Adult women									
ILCR	2.20E-06	6.90E-05	3.40E-05	3.20E-06	3.10E-04	9.70E-05	8.60E-07	1.10E-04	3.00E-05
HI	0.1	2.2	0.9	0.1	6.9	2.2	0.0	2.3	0.7
Male children									
ILCR	3.10E-07	4.70E-05	1.90E-05	4.49E-06	1.90E-05	1.10E-05	5.70E-06	6.70E-06	6.20E-06
HI	0.1	4.2	1.9	0.4	3.2	1.5	0.5	0.7	0.6
Female children									
ILCR	1.80E-06	2.50E-05	1.30E-05	2.20E-07	1.80E-05	1.00E-05	7.00E-06	7.00E-06	7.00E-06
HI	0.2	2.2	0.9	0.1	1.8	1.1	0.7	0.7	0.7

* Group's estimated risk is significantly greater than estimated risks for other sub-groups from the same lake.

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Commission did list cancer risks from environmental, diet, and genetic factors as a priority (EEA, 2004). USEPA uses an ILCR risk range of $1x10^{-6}$ to $1x10^{-4}$, and an HI < 1.0 (USEPA 1989, USEPA 1991(a)). 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 othercontrols.

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 $1.0E^{-06}$; 53 % of the mean HIs were greater than 1.0. For example, the mean site specific ILCR ($1.2E^{-04}$) 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 atthegreatest 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 in Lake Pamvotis, adult men are the sub-population at greatest risk. For *S. aristotelis*, the mean site specific ILCR for the general population ($1.80E^{-05}$, max.=$1.1E^{-04}$) and adult sub-populations (range=$1.40E^{-05}$- $3.0E^{-05}$) exceed the USEPA's point of departure for cancer risk; however, the mean ILCRs are below the level where the USEPA would likely require remedial action beyond some form of institutional control such as risk communication and / or consumption advisory. However, maximum ILCR values exceed this level.

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.

In both lakes, primary drivers for risk were consumption values (particularly those of men), and concentrations of PCBs and DDT related compounds present in fish tissues (low recoveries of DDD may underestimate risks from DDT and associated breakdown products). While the concentrations of halogenated compounds in the fishes we studied in Greece were lower than those reported for some U.S. sites (USEPA, 1991b; Grimes, 1994 and 1995), the substantially higher consumption rates of the local populations studied in Greece make health risks associated with these contaminant levels meet or exceed those at sites in the US where risk management actions have been implemented to protect human health.

As there is a need for restricted information on fish consumption data and human health risks associated with the consumption of fish tissues contaminated with

Virginia Journal of Science, Vol. 56, No. 3, 2005 https://digitalcommons.odu.edu/vjs/vol56/iss3

organochlorine pesticides in Greece, and published reports for the same in EU countries, our investigation is the first such study for Greece, and second for an EU country. Although there are studies indicating chemical loads for waters and sediments in some areas of the Mediterranean, no published reports have linked these chemical loads to human health effects. There also appears to be few studies surveying local fish consumption habits for the purpose of developing inputs to risk assessment models.

Pesticide concentrations in samples of fishes from EU members have not been reported as of the pesticide residue committee meeting in May, 2004 (Brown, 2004). Likewise, there are no adequate EU toxicity data for about 75 % of 3,000 substances in use; and no adequate EU ecotoxicity data for 50-75 % of the 3,000 priority High Production Volume Chemicals (HPVC) reviewed by the EU (EEA and UNEP, 1999). EEA (2004) and EEA and UNEP (1999) indicated there were significant gaps in toxicity and exposure data, and announced a major lack of human health and exposure data for these priority chemicals. To date, approximately 400 risks assessments have been conducted by EU member states; however, none are made available to the public as they are listed as confidential data (EEA and UNEP, 1999).

This study shows that chemicals from industrial and agricultural use over the past 50 – 60 years have come to rest in the rivers, lakes and other water bodies of Greece. Albanis (1992) found 2,4-D was found in Greek rivers and streams at every location tested between May and August, and that 17 % of applied 2,4-D ended up in water. Due to their environmental persistence and lipophilic nature, these compounds are bioaccumulating in the regional food chains and place the health of humans, as well as other (e.g. wildlife) top consumers on those food chains at potentially significant risk. This scenario not only opens the door to human health risk assessment issues, but also to environmental risk assessment for the aquatic and terrestrial biodiversity in the country. In a report of the OECD Project on pesticide aquatic risk indicators, OECD (2002) reported that the projects first focus on aquatic risks was a logical starting point as it would be simpler and a reasonable first step toward a comprehensive suite of risk indicators for assessment of terrestrial and human health risk.

The size and nature of populations at risk from contaminated fishes in Greece are unknown. Since exposure varies considerably under different circumstances, and the risk assessment process contains inherent uncertainties (USEPA 1991a), we concur with WHO (1999) which strongly encourages responsible authorities in countries to characterize risk using site-specific exposure scenarios and not default values. Use of USEPA default values would have underestimated the risk to Greek populations based on IR alone. Despite the use of maximum chemical concentrations in most cases, risk values in our study should be considered conservative as they focused only on identifiable halogenated organic compounds and did not look at risk contributions from non-halogenated organics, 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 studies in order to avoid risks. 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).

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