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Autor: Haldimann, Max / Blanc, Annabelle / Dudler, Vincent

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Mercury concentration in perch and whitefish from Swiss lakes

Max Haldimann, Annabelle Blanc and Vincent Dudler
Swiss Federal Office of Public Health, 3003 Bern, Switzerland

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Introduction

Mercury (Hg) is considered to be a contaminant of continuing concern. The major natural source of mercury is the degassing of the earth's crust. Industrial activities contribute a considerable part to the global emission of mercury to the atmosphere as well (1). Elemental mercury in the atmosphere is oxidized by ozone or hydrogen peroxide to soluble forms, e.g., Hg^{2+}, and deposited by rain onto soil and water (2). Its presence and behavior in aquatic systems is of ecological importance. Local variations of the mercury concentrations are considerable, especially in coastal sea water and in lakes or rivers where mercury is associated with suspended material. Hg^{2+} forms organometallic compounds. The most numerous are those of the type RHgX, where R represents the organic moiety and X may be one of a variety of anions. Hg^{2+} is converted to methyl mercury in sediments in fresh and ocean waters, e.g., by methyl cobalamine compounds that are produced as a result of bacterial synthesis (3). Fish absorb methyl mercury as they feed on aquatic organisms. Originating from plankton, methyl mercury accumulates in the aquatic food chain (4). Maximum concentrations are found in both freshwater and marine fish at the highest trophic levels. Predatory fish such as shark and swordfish may have average values of total mercury in edible tissues well above 1000 µg/kg (5), whereas pike from Swiss lakes does not normally exceed this concentration (6). Elevated mercury levels (>1000 µg/kg) in pike were found in Finnish reservoirs (7).

Methyl mercury is the predominant mercury species in fish. Methyl mercury to total mercury ratios approach unity in muscle tissue of higher food chain fish residing in waters that are relatively uncontaminated with mercury. Freshwater fish such as whitefish exhibit high methyl mercury to total mercury ratios as well (8).

Mercury toxicity depends on its chemical form, alkyl mercury compounds being the most toxic. Methyl mercury is lipophilic and penetrates cell membranes. In general, the effects of exposure to organic mercury are primarily neurological (9).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended a provisional tolerable weekly intake (PTWI) of mercury of 5 µg/kg body weight (bw), of which no more than 1.6 µg/kg bw weight should be methyl mercury (10). Total mercury in fishery products is regulated by the European Commission Decision 93/351 (11). The mean total content of mercury in edible parts of fish must not exceed 0.5 mg/kg of fresh products. The limit value is, however, increased to 1 mg/kg in certain predatory species. Switzerland has adopted essentially the same regulation (12).

Despite its excellent sensitivity, the use of ICP-MS for the determination of mercury in fish samples digested by nitric acid has been restricted because of memory effects and erratic signals (13). Although the limitations can be overcome by cold vapor generation or flow-injection (14), a rapid and robust method has been developed using tetramethylammonium hydroxide (TMAH) as a tissue solubilizer. TMAH solubilization was previously used as a sample preparation method for gas chromatographic determination of mercury (15). Species interconversion during the pretreatment of the fish samples with TMAH is of the order of only a few percent (16).

Human exposure to methyl mercury is primarily through the consumption of fish. Although most people's fish consumption does not cause adverse health effects, some fish may contain methyl mercury at sufficiently high levels to be a concern. The purpose of this study was to provide recent data on total mercury concentration in significant species of freshwater fish intended for human consumption and thereby identify sites with high fish-mercury concentrations. As a basis for food safety recommendations, it is important to have reliable up-to-date information on the levels and distribution of total mercury in individual fish. European perch *(Perca fluviatilis),* roach *(Rutilus rutilus)* and whitefish *(Coregonus* spp.) from Swiss lakes and lakes bordering Germany and France were analyzed. These species are among the principal fishing products. This paper presents the results of a survey of total mercury in 168 fish samples. The mercury measurements were part of a monitoring program focused on polychlorinated contaminants, particularly dibenzofurans (PCDF) biphenyls (PCB) and dibenzo-p-dioxins (PCDD) in fish (17).

Material and methods

Fish samples

The perch, roach and whitefish samples were collected from September to December 2001 by the respective fishery authorities of the lakes (Figure 1). From 5 to 34 fishes were obtained from each lake, with approximately equal numbers of males and females per species. The sample groups were comparatively homogenous with respect to length. The length, weight, and location of collection were recorded for each fish. The fishes were dissected, the skin was removed and representative tissues were cut into small pieces. The subset of the filet pieces was put aside and

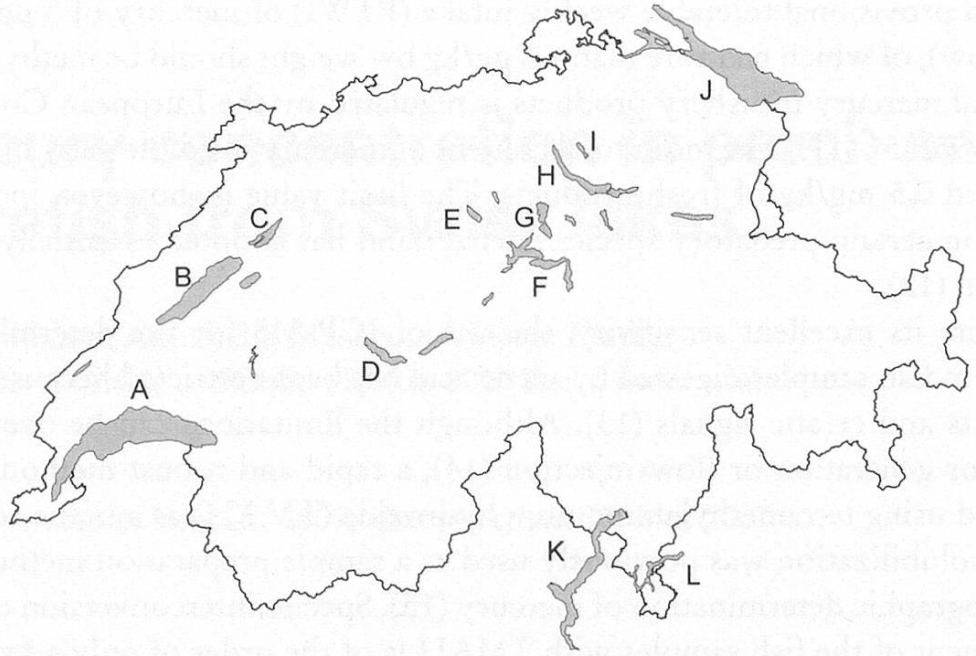

Figure 1 **Fish sampling locations for selected species: Lake Geneva (A), Neuchâtel (B), Biel (C), Thune (D), Sempach (E), Lucerne (F), Zug (G), Zurich (H), Greifen (I) and Constance (J). No samples were obtained from Lakes Maggiore (K) and Lugano (L)**

stored in a deep freezer at –20 °C. After thawing, the samples of fish tissues were cut into small pieces (approximately 0.5 cm cubes) on a plastic cutting board using a ceramic blade knife. The composite fish samples were freeze-dried for 48 h. Freeze-dried fish tissue can be homogenized more effectively than wet tissue (8). Subsequently, the dried samples were thoroughly milled into a homogenous sample using a knife mill (Grindomix GM 200, Retsch, Germany).

Pulverized freeze-dried fish tissue samples (0.3 g) and 2.5 ml of 25 % analytical grade TMAH solution (TAMA Chemicals, Japan) were placed in a graduated 50 ml polypropylene tube and heated in an oven at a fixed temperature of 90 °C for 3 h. After cooling, the sample solubilization solutions were made up to volumes of 50 ml by addition of ultra-pure water (18 MΩ, Easy Pure LF, Barnstead, USA) and then centrifuged for 5 min at 5100 rpm (Labofuge 400, Heraeus, Germany). The final dissolution for the measurement was made with 500 µl of the supernatant and 1500 µl 1.2 % TMAH solution.

Calibration solutions and reference materials

The stock solution of methyl mercury was prepared by dissolving methyl mercury (II) chloride (Pestanal®, Riedel-de Haën, Germany) in analytical grade 2-propanol (Riedel-de Haën) to obtain a solution of 100 µg ml^{-1}. The calibration solutions were prepared by appropriate dilution of the methyl mercury chloride

stock solution with 1.2 % TMAH solution to obtain mercury levels of 0.5, 1, 2 and 3 µg/l. Two certified reference materials were used to validate the proposed method. DORM-2 (dogfish muscle) and BCR-422 (cod muscle) were obtained from the National Research Council of Canada (NRC) and the Community Bureau of Reference (BCR), respectively. In addition, canned fish test material (tuna) was obtained from FAPAS®. The FAPAS sample was prepared in the same way as described above.

Instrumentation

The sector field-ICP-MS instrument used in this work was an Element 2 (Thermo-Finnigan, Germany), equipped with a mini-cyclonic glass spray chamber with a central tube (Twinnabar, Glass Expansion, Australia) for a better filtering effect and a Sea Spray nebulizer (Ar30-07-FSS04, Glass Expansion) operating at a sample uptake of 0.3 ml/min and sample-gas (Ar) flow ranging from 0.9–1.0 l/min (Auxiliary gas flow 0.6 l/min). The plasma-gas flow was 16 l/min at a power of 1.2 kW. The mercury isotope ^{202}Hg was monitored using take-up and wash times of 2 min and 10 min, respectively. Blanks, standards and samples were measured using this procedure.

Statistical evaluation

The two-sample t-test was performed to compare the mean mercury concentrations for the two principal species perch and whitefish originating from the same lake (5 cases). Pearson correlation coefficients (r) were computed to examine potential relationships between mercury concentration and biometric data.

Results and discussion

Method and quality control

TMAH was considered as the most appropriate tissue solubilizer, as it was able to dissolve the samples almost completely under mild conditions. Following the solubilization of the fish samples in TMAH, the fraction of inorganic mercury was expected to be bound to soluble proteins or other molecules and therefore the residual sample matrix was not analyzed for insoluble mercury (18). Compared with conventional sample preparation techniques for mercury analysis, this method is relatively simple and fast. Methyl mercury chloride has a covalent Hg-Cl bond, thus its use as calibration standard emulates the chemical form of mercury in fish (19). The Sea Spray nebulizer that was designed to produce aerosol from solutions with a high content of total dissolved solids (20) has proven to be effective for solubilized solutions; hence no instabilities were observed.

Quality control measures for each analytical batch included duplicates of two different standard reference materials with certified mercury concentrations. In addition, the laboratory participated in a proficiency testing program (FAPAS®),

which is a food analysis performance assessment scheme (21). The quality control results, summarized in Table 1 were satisfactory, as the 95 % confidence intervals of both reference materials largely overlap with the corresponding intervals of the measurements. The performance in the FAPAS® proficiency test was considered satisfactory because an individual z-score of 1.1 was obtained (Table 1). The positive z-score value indicates that the method, including sample preparation, was not prone to mercury losses. In addition, the results in Table 1 suggest that the method yields results for total mercury and is not selective with regard to methyl mercury. Duplicate analysis for mercury was completed on homogenized fish tissue during the analytical runs and showed a 93 % agreement between samples on average. The between-run precision as inferred from the BCR 422 sample was about 4 % (Table 1).

Table 1
Quality control results summarized for all analytical batches (±half width of the 95 % confidence intervals)

Reference material	*n*	*Certified or target values*		*Measured total mercury (µg/kg)*
		Total mercury (µg/kg)	*Methyl mercury (µg/kg)*	
BCR CRM 422, cod	6[1]	559±16	430±60	534±20
NRC DORM-2, dogfish	5[1]	4640±260	4470±320	4660±130
FAPAS®, canned tuna	3	397±146[2]	no data	476±19

[1] sets of results (each of 2 replicates)
[2] assigned value with z-scores of −2 and +2, based on the results submitted by the participants in food analysis performance assessment scheme (FAPAS®).

Edible tissue mercury concentration and geographical variation

Table 2 summarizes the mean total mercury concentrations found in each composite sample with its characteristics (species, weight and sizes of fish) and location of sampling. By species, the average mercury concentration (wet weight) ranged from 24.8 to 120 µg/kg for whitefish and from 77.5 to 173 µg/kg for perch. Only one composite sample was available for roach.

The results for all samples did not exceed the maximum legal limit of 0.5 mg/kg (wet weight) for these species. The highest mercury concentrations were found in perch and whitefish harvested from Lake Biel, probably as a result of high microbial activity, i.e., microbial synthesis of methyl mercury from its inorganic precursor (22). Within species, mercury concentrations varied according to the sample sites. Among species, significant differences between perch and whitefish were found (Figure 2). In fact, higher mercury concentrations were measured in perch than in whitefish, if pairs originating from the same lake were compared ($p=0.02$). The differences in mercury concentrations indicate the higher trophic position of European perch, which is a predatory species that feeds on fishes and invertebrates. These findings agree with a previous study on whitefish and perch collected from several Swiss lakes (23).

Table 2
Total mercury (Hg) concentrations in edible tissue (wet and dry mass) of whitefish (*Coregonus* spp), perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*), collected from lakes in Switzerland

Fish Species	*Sampling Site*	*Harvest (t)*[1]	*Sample Size*[2]	*Weight (g)*[3]	*Length (cm)*[3]	*Hg (µg/kg) dry mass*	*Hg (µg/kg) wet mass*
Whitefish	Lake Biel	96	10	259±22	30.7±1.2	457	120
Perch	Lake Biel	15	10	149±72	23.6±2.9	746	173
Whitefish	Lake Constance	265	10	283±62	32.3±2.7	239	64.3
Perch	Lake Constance	78	34	79.6±21	18.9±1.4	359	77.5
Whitefish	Lake Geneva	67	10	552±45	40.3±1.6	118	33.2
Perch	Lake Geneva	165	14	64.0±16	17.4±1.9	524	114
Whitefish	Lake Greifen		5	640±114	40.4±1.7	262	62.9
Whitefish	Lake Neuchâtel	201	10	304±96	33.4±2.7	191	56.6
Perch	Lake Neuchâtel	51	10	93.0±96	18.3±4.7	376	82.4
Whitefish	Lake Sempach	71	10	95.5±14	26.4±1.2	113	24.8
Whitefish	Lake Thune	11	10	237±39	30.4±1.3	327	85.0
Whitefish	Lake Lucerne	113	10	75.7±8.8	21.9±1.1	224	50.2
Whitefish	Lake Zug	9	10	319±99	31.6±2.7	207	45.7
Roach	Lake Zurich	38	5	372±220	27.4±6.5	241	53.2
Whitefish	Lake Zurich	138	5	390±33	34.4±1.1	163	36.7
Perch	Lake Zurich	50	5	274±207	27.2±6.8	559	113

[1]Mean harvest data from 2002/3 (Swiss Agency for the Environment, Forest and Landscape 2005), resulting in a weighted mean $\bar{x}_w = \Sigma\, w_i x_i = 70$ µg/kg; where w_i is the ratio between individual and total harvest and x_i the mercury concentration (wet mass).
[2]Number of individual fishes per composite sample
[3]Mean value±standard deviation

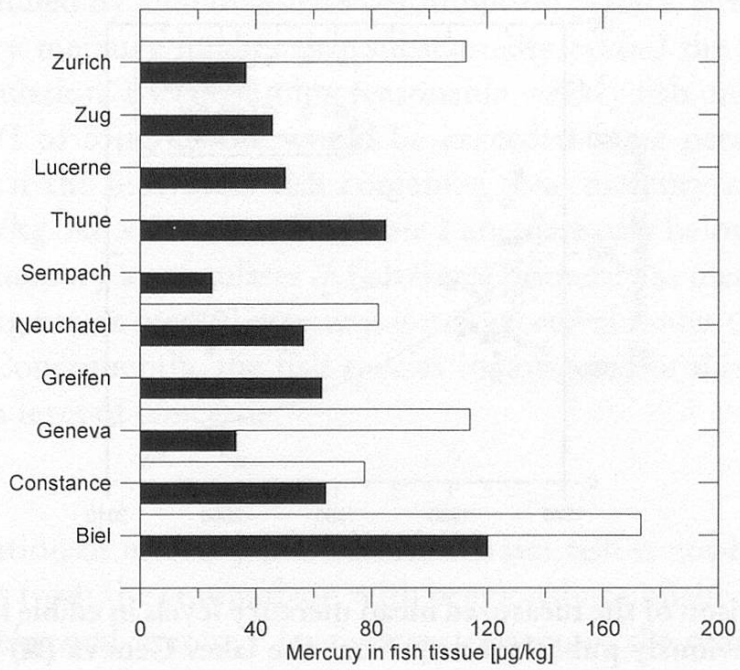

Figure 2 **Total mercury concentrations in tissue of perch (white bars) and whitefish (black bars) from Swiss lakes**

The changes in fish-mercury concentrations among samples collected across different lakes are due to either spatial or sample characteristic effects or some combination (Figure 2). The fish-mercury concentrations in this study represent average values from subsets, which consist of samples with differing characteristics, such as ecotype, age or sizes of fish. Ecotypes feed on different organisms and large-size fish may accumulate more mercury. Mercury concentrations in fish are known to increase with age due to cumulative exposure (24), but fish ages were not recorded for the caught populations. Fish lengths and weights can be used as surrogates for age, and positive relationships between mercury concentrations and both size and age were reported for perch (24, 25). Due to the limited number of observations (composite samples) in this study, the mercury concentrations were not positively related to both length and wet weight in whitefish and perch ($r=0$; $0.05<p<1$).

Development of the fish mercury concentration

To put the observations of fish tissue mercury levels into perspective, comparable measurements from previous and recent studies were compiled (22, 23, 26, 27). Available data regarding mercury concentrations in perch and whitefish from the lakes Geneva and Biel are shown in Figure 3. Although perch and whitefish of variable size are included in the data presented in Figure 3, a distinction for the mercury concentrations between the two species is visible. This distinction remains even if the time aspect regarding the decline of mercury in fish from Lake Geneva is taken into consideration.

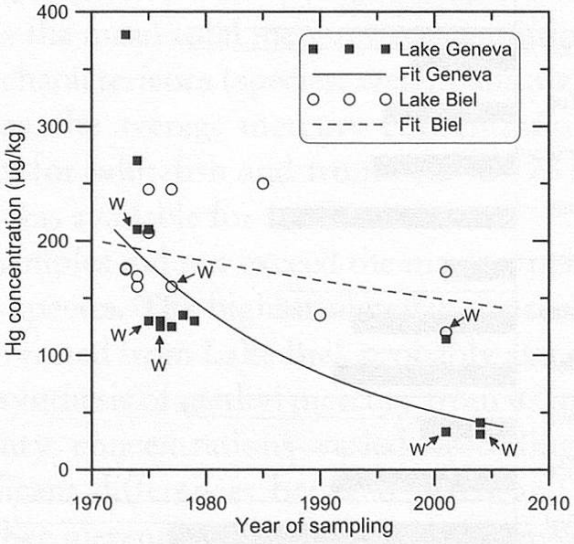

Figure 3 **Comparison of the measured mean mercury levels in edible fish tissue (muscle) with previously published data from the lakes Geneva (■) and Biel (O). The dots denoted either with "w" or without a letter are mean values of mercury concentrations in whitefish and perch, respectively. The fits (ln[Hg]=a+b • Year) to the data corroborate the difference in fish mercury decline for the two lakes**

The reduced concentrations in fish from Lake Geneva may be a result of initiatives for controlling releases and limiting use of mercury (28). Contrary to Lake Geneva, the corresponding data from Lake Biel do not show a comparable decrease in mercury levels over time.

In this study, no samples were obtained from the lakes Maggiore and Lugano bordering Italy (Figure 1). Published data show, however, that the mercury level in whitefish from Lake Maggiore (117 ± 10 µg/kg) falls in the range listed in Table 2 and has remained constant over the past six years (29).

Risk assessment

The total fish intake that consists of fresh, frozen and processed fish varies greatly throughout Europe (30). FAO statistics show the highest annual per capita consumptions to be in Norway (50 kg), Spain (41 kg) and France (29 kg), whereas Germany (14 kg) and Switzerland (8 kg) rank among the lowest in Europe (31). Perch, roach and whitefish are the most frequently caught species in Swiss lakes. The annual harvest yields approximately 1500 t, which, however, represents only a small fraction (2.5 %) of the Swiss per capita consumption (32). The estimated average weekly consumption of local freshwater fish by the Swiss population is therefore only about 4 g. As it is imperative to consider fish that is actually being consumed, an overall mean mercury tissue concentration of 70 µg/kg was calculated weighted with the respective harvests (Table 2). As a result, the weekly per capita mercury exposure from these species was about 0.3 µg, i.e., a dietary exposure much lower than that recommended by JECFA, assuming any body weight. The average population, however, includes both fish eaters and non-fish eaters. Exposure to fish-mercury is determined by an individual's consumption pattern. Frequent fish eaters could have dietary mercury intakes that substantially exceed the average mercury intake of the population. By consuming reasonable weekly fish meals of 200 g (30), the JECFA PTWI of 5 µg/kg bw would be exceeded for a person with a body weight of 60 kg, if the individual fish contained total mercury concentrations in excess of 1500 µg/kg. All values listed in Table 2 are markedly below this concentration. Given that mercury accumulates in fish tissue primarily as methyl mercury, the PTWI of 1.6 µg/kg bw, as methyl mercury, is not exceeded under the same assumptions as before. Consequently, the fish species investigated in this study were not contaminated to a level of concern.

Conclusion

The accumulation of mercury in Swiss freshwater fish is not higher than what one would expect from the comparison with previously published data. Although no significantly elevated mercury levels were observed, the data obtained from this monitoring program indicate that mercury contained in edible portions of fish tissue can vary greatly depending on the species collected and the location of the harvest. In principle, comprehensive risk assessments should take into account

the geographic location. The mercury risk depends on the dietary habits of the consumer and sources of contaminated fish. The mercury exposure level estimated in this study does not pose a health threat to average consumers of freshwater fish. In contrast, people who consume high amounts of contaminated marine fish may be more exposed to mercury and are therefore at greater risk of adverse health effects. Public health food policies should strike a balance between the nutritional benefits of fish and the potential risk from exposure to contaminants such as mercury compounds.

Acknowledgment

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Summary

The purpose of this study was to determine the concentrations of mercury in the edible portion of fish tissue and to estimate mercury exposure from the consumption of European perch, whitefish and roach collected from selected lakes in Switzerland. The samples were grouped by species and location of collection and were analyzed as composites. The concentration of total mercury was determined using sector-field ICP-MS after tissue solubilization with tetramethylammonium hydroxide. The concentrations ranged from 24.8 to 120 µg/kg and from 77.5 to 173 µg/kg in the tissue of whitefish and perch, respectively. The single roach composite sample had a concentration of 53.2 µg/kg. Differences were noted in the concentration of mercury depending on the location of collection and the species examined. Perch contained higher concentrations of mercury when compared with whitefish from the same lake. The consumption of fish with mercury levels found in this study is not likely to result in exposures of concern. Even frequent consumers are not at risk from such mercury levels. Provided that normal quantities of perch and whitefish are consumed once a week, the dietary mercury intake would fall below the provisional tolerable weekly intake (PTWI).

Zusammenfassung

Der Zweck dieser Studie war die Bestimmung von Quecksilber im essbaren Anteil von Fisch und die Abschätzung der Quecksilberbelastung, die sich aus dem Verzehr von Egli, Felchen und Rotauge ergibt. Die Proben wurden entsprechend der Fischart und der Herkunft aus ausgewählten Seen der Schweiz als Mischproben analysiert. Die Gesamtkonzentration von Quecksilber wurde nach Auflösen des Gewebes in Tetramethylammoniumhydroxid mittels Sektorfeld-ICP-MS bestimmt. Die Konzentrationen reichten von 24,8 bis 120 µg/kg für Felchen, beziehungsweise von 77,5 bis 173 µg/kg für Egli. Die einzige Mischprobe für Rotauge wies eine Konzentration von 53,2 µg/kg auf. Abhängig vom See, aus welchem die Fische her-

kamen und von der Fischart, ergaben sich Unterschiede in der Quecksilberkonzentration. Im Vergleich zu Felchen war die Quecksilberkonzentration in Egli stets höher, falls die Proben aus demselben See stammten. Der Verzehr von Fischen, welche die in dieser Studie gemessen Gehalte aufweisen, führt nicht zu einer besorgniserregenden Belastung mit Quecksilber. Selbst Konsumenten, die häufig Fisch verzehren, gehen kein erhöhtes Risiko ein. Unter der Voraussetzung, dass nicht übermässig hohe Mengen an Egli und Felchen konsumiert werden, liegt die Quecksilberaufnahme unterhalb der vorläufig tolerierbaren wöchentlichen Aufnahmemenge (PTWI – provisional tolerable weekly intake).

Résumé

La teneur en mercure dans la partie comestible de poissons provenant de lacs suisses (perche commune, corégones et gardon) a été déterminée dans cette étude et l'exposition au mercure a été estimée à partir de leur consommation. Les poissons ont été groupés par espèces et lieu de pêche puis analysés comme échantillon composite. La concentration totale de mercure a été déterminée par spectrométrie de masse à secteur magnétique ICP-MS après solubilisation des chairs par de l'hydroxyde de tétraméthylammonium. Les concentrations mesurées se répartissent entre 24,8 et 120 µg/kg pour les corégones et entre 77,5 et 173 µg/kg pour la perche commune. L'unique échantillon composite de gardon montre une concentration de 53,2 µg/kg. Des différences dans la concentration de mercure sont observées en fonction du lieu de pêche et de l'espèce. La perche commune a une teneur en mercure plus élevée que les corégones d'un même lac. La consommation de poisson ayant une teneur en mercure semblable à celles observées dans cette étude ne pose pas de risque sanitaire. A ce niveau de contamination, même une consommation régulière ne pose pas de problème. En estimant qu'une quantité normale de perche et corégones soit consommée une fois par semaine, la quantité de mercure ingéré reste inférieure à la valeur de consommation hebdomadaire tolérable provisoire (PTWI – provisional tolerable weekly intake).

Key words

freshwater fish, mercury, perch, roach, sector-field ICP-MS, tissue solubilization, whitefish

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Corresponding address: Max Haldimann, Swiss Federal Office of Public Health, Division of Food Science, Food Chemistry Section, 3003 Bern, Switzerland,
e-mail: max.haldimann@bag.admin.ch