Serum concentrations of *beta*-hexachlorocyclohexane in groups of the Italian general population: a human biomonitoring study

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Summary. Because of its persistence and toxicological profile, *beta*-hexachlorocyclohexane (β-HCH) has been proposed for inclusion in the Stockholm Convention on persistent organic pollutants (POPs). Although the use of technical HCH, which is the primary source of β-HCH in the environment, has been banned in the EU in 1978 and progressively at a global level, β-HCH is still detectable in the general environment worldwide. Human exposure mostly occurs *via* food and may be of concern in areas where illegal use and/or improper disposal of stockpiles occurred and locally grown food is consumed. Exposure of the Italian general population to β-HCH has been poorly characterised. Lack of human biomonitoring data severely hinders the ability to interpret potential increases in exposure related to situations of environmental risk. We carried out a human biomonitoring study aimed to provide baseline information on background exposure of the Italian general population to this pollutant. For this purpose, we analysed 116 serum samples from groups of subjects of both sex from the general population residing in three Italian towns at different latitudes. Serum concentrations of β-HCH resulted to be comprised between 1.64 and 300 ng/g fat, with a median value of 18.0 ng/g fat and a 90th percentile of 65.9 ng/g fat. The serum concentrations detected are in line with those detected in most Western European countries.

Key words: beta-hexachlorocyclohexane, Italy, human serum, general population, human biomonitoring.

Riassunto *(Livelli di* beta*-esaclorocicloesano in gruppi della popolazione generale italiana: uno studio di biomonitoraggio).* Il *beta*-esaclorocicloesano (β-HCH) è un inquinante ambientale a elevate persistenza e tossicità recentemente proposto per l'inclusione nella Convenzione di Stoccolma sui *persistent organic pollutants* (POPs). La sua presenza nell'ambiente è stata determinata dal vasto uso dell'esaclorocicloesano (HCH) tecnico, una miscela di diversi isomeri di cui il β-HCH rappresenta il componente a più elevata persistenza. Sebbene l'uso dell'HCH sia stato bandito nei Paesi dell'Unione Europea da molto tempo, il β-HCH è ancora presente nell'ambiente. L'esposizione umana avviene essenzialmente per via alimentare e può essere causa di rischio sanitario in aree contaminate, in particolare quando in queste vengano consumati alimenti di produzione locale. I dati di esposizione della popolazione generale italiana a β-HCH sono scarsi, e questo rende difficile rilevare e interpretare un eventuale incremento espositivo. Nel presente studio sono stati analizzati 116 campioni di siero di soggetti di ambo i sessi residenti in tre città italiane a diversa collocazione geografica. Le concentrazioni del β-HCH sono risultate essere comprese nell'intervallo 1,64-300 ng/g grasso, con una mediana di 18,0 ng/g grasso e un 90esimo percentile di 65,9 ng/g grasso. I valori riscontrati sono in linea con quelli generalmente osservati nei paesi dell'Europa occidentale.

Parole chiave: beta-esaclorocicloesano, popolazione generale italiana, siero, biomonitoraggio.

INTRODUCTION

Beta-hexachlorocyclohexane (β-HCH) has been recognised as a persistent organic pollutant (POP) of major and global concern for the environment and health. Its origin in the environment is due to the widespread use of technical hexachlorocyclohexane (HCH), a commercial mixture of five isomers produced during manufacture as a result of the photochemical chlorination of benzene: α-HCH (55-80%), β-HCH (5-14%), γ-HCH, (8-15%), δ-HCH (6-10%) and ε-HCH (1-5%) [1].

As the γ-HCH isomer, known as lindane, is the only isomer characterised by high pesticidal activity, technical HCH is subject to subsequent fractional crystallization and concentration to produce 99% lindane. This process has a very low yield (only 10-

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15%), producing 6-10 tons of the other isomers for each ton of lindane [2]. *Alpha*-HCH is the major by-product of the reaction (60-70%), followed by β-HCH (7-10%) [3].

Beta-HCH is the most persistent isomer in the environment (half-lives of 184 and 100 days for cropped and uncropped plots [4]), since its all equatorial configuration favours resistance to hydrolysis and photolysis [5]. It is also the predominant HCH isomer in animal tissues. In humans, its half-life in blood is of about 7.2 years [6].

Potential for moderate bioaccumulation of β-HCH is indicated by the n-octanol-water partition coefficient (K_{ow} = 3.78) and confirmed by the observations carried out on Arctic marine food webs. Birds and marine mammals, in particular, can accumulate β-HCH to higher levels than those observed for the other isomers [7-9].

The toxicological profile of β-HCH is characterized by neurotoxicity, hepatoxicity, and reported estrogenic effects in mammalian cells and laboratory mammals [10].

In humans, neurophysiological and neuropsychological disorders have been observed in workers exposed to technical HCH during pesticide or fertilizer formulation at reported β-HCH serum levels in the indicative range of 14-144 μg/g fat [4].

A possible correlation between human exposure to β-HCH and breast cancer has been investigated in several epidemiological studies. Most studies showed a weak — not statistically significant — correlation [4], while a significant association between high blood β-HCH concentrations and breast cancer in premenopausal women was reported by a Chinese study [11]. The International Agency for Research on Cancer (IARC) has classified β-HCH in Group 2B as possibly carcinogenic to humans [12]. EPA's Integrated Risk Information System (IRIS) lists β-HCH as a possible human carcinogen, on the basis of the incidence of hepatic nodules and hepatocellular carcinomas observed in male mice administered with β-HCH at a single dose level in the diet [7].

After almost forty years of extensive use worldwide, there has been a gradual replacement of technical HCH by lindane. No significant uses of technical HCH have been reported after 2000 worldwide. HCH, including lindane, has been included in the UNECE Protocol on POPs in 1998. Today, both lindane and technical HCH are totally banned within EU, and lindane and the other HCH isomers have been recently considered for inclusion in the UNEP POP Stockholm Convention [13].

Human exposure of the general population to β-HCH mainly occurs through food. The mean bioaccumulation factor for β-HCH in humans has been calculated to be 527 (range 310-744) [14], based on concentrations in human diets from several countries and corresponding levels in adipose tissue.

In areas where environmental contamination occurred and locally grown food is consumed, exposure via contaminated food may be of toxicological relevance. Concentrations of β-HCH far exceeding maximum values set by the European Economic Community [15] were detected in raw bovine milk samples from farms located in the Valle del Sacco area, central Italy, in 2005 [16]. The Sacco river is one of the main rivers of the Lazio region and runs north to south in an open and densely populated valley. Since the beginning of 1900 to the end of the '90s, industrial settlements located in the area produced a wide range of chemical products, including explosives, industrial chemicals and pesticides, in particular technical DDT and HCH. Over the years, industrial activities, and related improper waste disposal, determined a widespread environmental contamination by HCH isomers, as well as heavy metals, DDT and DDE, and PCBs, of areas located inside the industrial settlements as well as in outer neighbouring areas along the river. This contamination, at first detected in soil and water samples, entered the local food chains and affected farms located along the Sacco River, as shown by the contamination of raw milk samples.

Concerns of the local residential communities prompted local and regional sanitary authorities to set up a biomonitoring study aimed to assess if an incremental exposure to β-HCH had occurred.

The need to interpret the study results, also on the basis of background exposure data to β-HCH of the Italian population, proved the substantial lack of this kind of information [17]. In fact, exposure of the general population to β-HCH in Italy has never been systematically characterised and the only available literature data refer to human milk samples collected in a few Italian towns in the late '80s [18].

The present investigation aimed to identify baseline β-HCH concentrations in groups of the Italian general population by biomonitoring subjects residing in northern, central, and southern Italy. The study was carried out in the framework of human biomonitoring activities performed by our group at ISS and supported by the Italian Ministry of the Environment aimed at characterizing present levels of internal exposure to POPs, also in accomplishment of obligations for Parties identified by the UNECE Protocol on POPs [19].

MATERIALS AND METHODS

Selection and enrollment of subjects

Analysis of β-HCH was carried out on serum samples from subjects residing in Rome, Brescia, and Naples, enrolled in 2008-2009 within the framework of studies aimed at assessing human exposure to organochlorinated POPs already under the Stockholm Convention, or proposed for inclusion. All subjects were characterized by having resided in the area for at least 15 years, lack of professional exposure to organochlorinated pesticides and, for women, by not having breastfed in the last 15 years. A total of 116 subjects of both sexes were included in the present investigation, 54 individuals residing in Rome (age-range 23-56 years), 28 individuals in Brescia (age-range 30-60 years), and 34 in Naples (age range 21-63 years).

Prior to blood withdrawal, each participant signed an informed consent form and compiled a questionnaire aimed to gather information on dietary habits and other possible sources of exposure to organochlorinated pesticides, medical history, and reproductive/nursing history in women.

Analysis

An aliquot of 10 mL of each serum sample was fortified with ^{13}C-labelled β-HCH and allowed to rest overnight. Thereafter, samples were added with a mixture of formic acid and 2-propanol, sonicated, and extracted by manual shaking with n-hexane. The organic phase was removed after centrifugation, and the extraction process was performed two times. The organic fractions were mixed together into a centrifuge tube, added with concentrated sulfuric acid, shaken and separated by centrifugation. The extracts were reduced in volume, purified on activated neutral alumina, concentrated and transferred to 1-mL autosampler vials for quantification.

Instrumental analysis was carried out by ion trap mass spectrometry, using a Thermo Scientific PolarisQ GC-ion trap in the MS/MS mode. Data were processed using the XCALIBUR software. Recovery rates were in the range of 75-110%. Analytical reliability was warranted by the use of an in-house validated method [20]. Lipid determination (cholesterol, phospholipids, and triglycerides) was carried out by enzymatic methods [20].

Statistical analysis

Data were analyzed using common statistical techniques in order to characterize their statistical distribution. Non-parametric tests (Spearman ρ test, median test, Mann-Whitney U test) were used to investigate the association of β-HCH serum concentrations with sex, age and the geographical origin of the subjects (STATISTICA, version 6.0).

RESULTS AND DISCUSSION

A total of 116 subjects from the Italian adult general population were included in the study, who made up the following subgroups on the basis of sex, age, and geographical origin: 66 males and 50 females; 28 subjects from northern Italy, 54 from central Italy, and 34 from southern Italy; 47 in the age range 20-35 years, 45 in the range 36-50 years, and 24 in the range 51-65 years.

Beta-HCH was detected in 93% of the samples, at a limit of determination of 1.5 ng/g fat.

Characteristics of the enrolled subjects, and determined serum concentrations of β-HCH expressed using the median as a measure of central tendency are summarised in *Table 1*, together with pertinent means and standard deviations.

Beta-HCH concentrations of all the analyzed samples ranged from 1.64 to 300 ng/g fat, with a median value of 18.0 ng/g fat and a 90th percentile of 65.9 ng/g fat. Values comprised in the interquartile range 9.37-29.1 ng/g fat can be considered to be representative of β-HCH concentrations characteristic of the general population.

Median values of the subgroups are 17.3 and 18.0 ng/g fat for males and females, 25.1, 12.4 and 22.5 ng/g fat for North, Centre and South, and 10.6, 18.1 and 39.4 for subjects in the age ranges 20-35, 36-50, and 51-65 years respectively.

Analysis of the statistical distribution of β-HCH serum concentrations (N = 116) showed that the distribution was not normal (Shapiro-Wilk's test, $p << 0.01$) and the data were better described by a log-normal distribution. Log-normal distributions of data grouped by sex, geographic area, and age range are shown in *Figure 1*.

Data distributions by sex substantially overlap, this confirming the non statistically significant dif-

Table 1 | *Concentrations (ng/g fat) of beta-hexachlorocyclohexane in groups of the Italian general population*

Characteristic of subjects	N	Median	Min-Max	Mean (SD)	10th Percentile	25th Percentile	75th Percentile	90th Percentile^{b}
All	**116 (108)^{a}**	**18.0**	**1.64-300**	**29.0 (40.0)**	**5.25**	**9.37**	**29.1**	**65.9**
Subgroups								
Males	66 (62)	17.3	1.64-300	31.5 (47.6)	5.25	9.61	29.9	—
Females	50 (46)	18.0	1.81-136	25.7 (27.3)	4.98	8.54	28.7	—
Northern Italy	28 (27)	25.1	2.17-207	41.5 (46.1)	5.25	12.3	—	—
Central Italy	54 (49)	12.4	1.64-118	18.6 (19.9)	4.96	7.96	21.4	—
Southern Italy	34 (32)	22.5	1.81-300	35.2 (53.5)	5.60	11.1	30.0	—
Age 20-35	47 (42)	10.6	1.81-118	14.7 (17.1)	4.83	6.60	18.0	—
Age 36-50	45 (42)	18.1	1.64-102	24.0 (21.0)	4.48	9.65	29.9	—
Age 51-65	24 (24)	39.4	12.6-300	66.5 (68.2)	21.4	26.0	—	—

^{a}*Number of samples with concentrations above limit of determination.*
^{b}*Only percentiles calculated on a sufficient ($N (1-p) \geq 8$) number of subjects (N) are shown.*

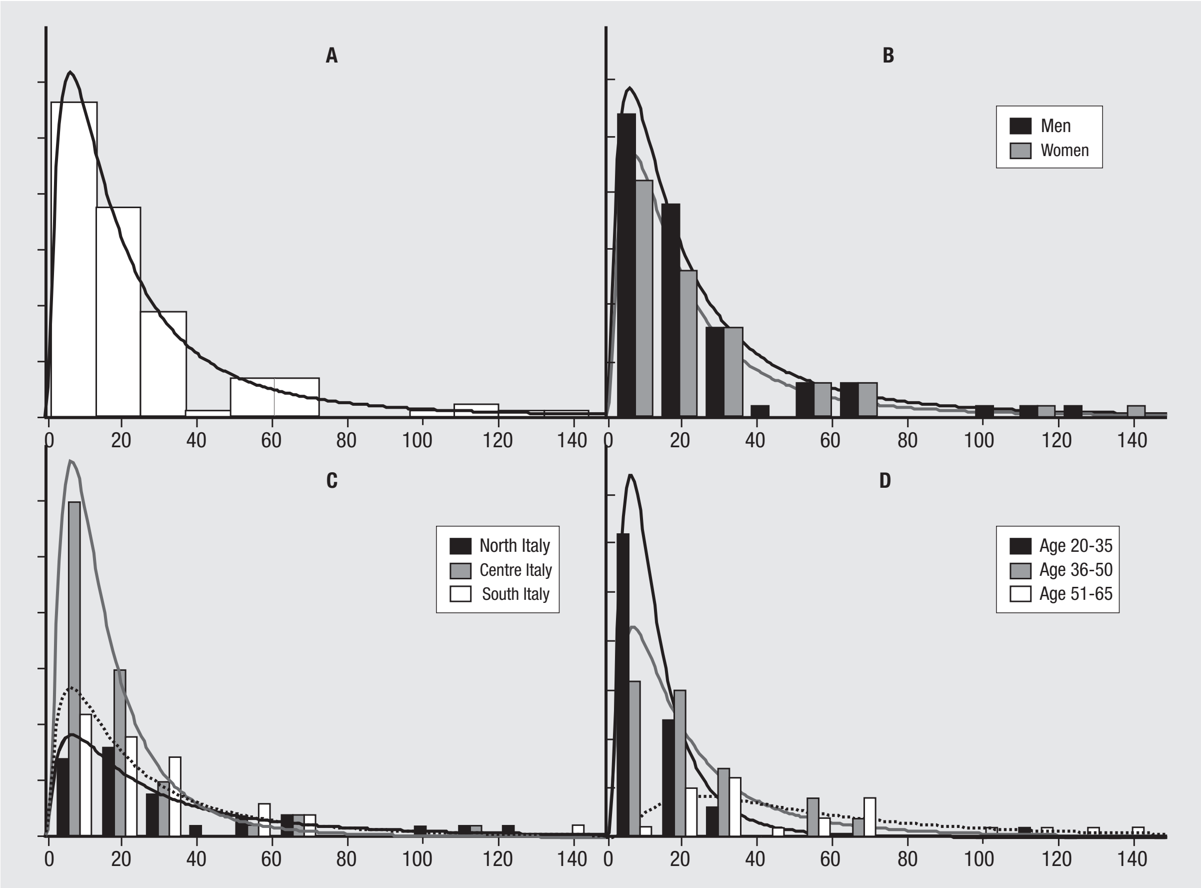

Fig. 1 | *Log-normal distributions of beta-HCH serum concentrations (ng/g fat) of all the subjects enrolled (A) and of subgroups of this population grouped according to gender (B), geographic origin (C), and age (D).*

ference between β-HCH concentrations in the two groups (Mann-Whitney U test, $p = 0.81$).

Data grouped by geographical area (northern, central, and southern Italy) show similar β-HCH serum concentration distributions for subjects from northern and southern Italy, whereas data from central Italy show a lower median value and a narrower distribution; the median test confirms a statistically significant difference ($p = 0.03$) between the three groups. An element that has to be considered in interpreting these results is that the subjects from central Italy are younger than those from the North and

Table 2 | *Concentrations of beta-hexachlorocyclohexane in groups of the general population of European and non-European countries*

Sampling year	Country	Sample type	Concentration (ng/g fat)	N. of donors	Reference
European countries					
2000	Czech Republic	milk	56[a]	43	[26]
1996	Finland	plasma	17.5[b]	143	[27]
1995-1997	Germany	milk	40[a]; (4-250)[d]	246	[28]
2006	Germany	milk	20.9[a]	523	[29]
1987	Italy	milk	163[a]	27	[18]
2008	Italy	serum	18.0[c]	116	Ingelido *et al.*, present study
1994	Kazakastan	milk	2210[a]	76	[30]

(continues)

Table 2 | *(continued)*

1996-1997	Northern Russia	milk	183[a]; (57-525)[d] 401[a]; (14-1202)[d]	98	[31]
1995-1996	Norway	plasma	15[b]	60	[27]
2000-2001	Norway	milk	Tromsø-10 [a] Oslo GRÜ-14[a] Oslo SNO-6[a]	10	[32]
2004	Poland	serum milk	3.9[a] 13.3[a]; (1.5-34.1)[d]; 11.0[c]	18 22	[33]
1997	Portugal	serum	9075[a]; (<800-109750)[d]	40	[23]
2001	Portugal	serum	2075[a]; (<800-26000)[d]	40	[23]
2001	Portugal	serum	Coimbra 400[a]; (<800-875)[d] Verride 550[a]; (<800-3325)[d] Ereira 825[a]; (<800-11075)[d]	44 70 89	[24]
2005	Romania	serum	923[c]; (<38-11690)[d]	142	[22]
1996	Russia	plasma	425[b]	51	[27]
1994	Spain	serum	2300[a]; max 58525	541	[34]
1995	Sweden	plasma	22.5[b]	40	[27]
1996-1997	Sweden	serum	51.1[c]; 62.7[a]; (7.4-744)[d]	205	[35]
1996-1999	Sweden	serum	9[a]; (3-60)[d]	325	[36]
1997-1998	UK	milk	68[a]; 50[c]; (<8-750)[d]	61	[37]
2001-2003	UK	milk	17[c]; (1.2–1500)[d]	54	[38]
2003	UK	serum	12[c]; (<0.68-80)[d]	151	[39]
1993-1994	Ukraine	milk	731[c]; (137-2387)[d]	197	[21]
Non-European countries					
2002-2003	Australia	milk	21[a]	157	[40]
1993	Australia	milk	345[a]; 184 [c]; (1-4400)[d]	54	[41]
1997	Brazil	serum	99.3[a]; (21.25-627.5)[d]	36	[42]
1992-2002	Canada	siero	57.5[a]; 20[b]	385	[43]
1996-1998	Cina	siero	5065[c]; (279-28.800)[d]	250	[44]
1999	Honk Kong	milk	950[a]	115	[45]
2002	India	milk	Dalian = 1400[a]; (130-7200)[d] Shenyang = 550[a]; (110-2200)[d]	20 20	[46]
2004-2006	India	milk	New Delhi = 240[a]; (4.2-1600)[d] Mumbai = 210[a]; (6.1-1200)[d] Kolkata = 680[a]; (61-1900)[d]	21 26 17	[47]
2006	Iran	milk	Nour = 1610[a] Countryside of Nour = 1580[a] Noushahr = 4000[a] Countryside of Noushahr = 2340[a]	23 10 10 14	[48]
1998	Japan	milk	210[a]; (42-969)[d]	49	[49]
1999	Japan	siero	135[a]; (12.5-37.5)[d]; 125 [c]	41	[50]
1999-2000	Japan	siero	93.2[c]; (60.8-171.0) [d]	80	[51]
2006	Korea	siero	49.0[c] ; 58.8[a]; (16.6-134)[d]	40	[52]
1995	Mexico	siero	54.25[c]	133	[53]
1997-1998	Mexico	milk	61[a]; (3-181)[d]	60	[54]
2000	Mexico	siero	233[a]	60	[55]
2003-2005	Tunisia	milk	42[a]; (1-285)[d]	237	[56]
2003	Turkey	milk	149[a]	37	[57]
1999-2000	USA	serum	36.9[c]	426	[58]
2003-2004	USA	siero	14.0[a]	1297	[59]

[a] *Arithmetic mean;*
[b] *Geometric mean;*
[c] *Median;*
[d] *Range;*

the South. In fact, when applying the median test to the three age-related data subsets, no significant differences were found ($p > 0.09$) on the basis of geographical origin.

Distributions of data grouped by age show an increase of the median values and of the curve width with age, indicating that the oldest part of the population is characterised by the highest β-HCH concentrations observed, and also by the broadest range of concentrations. Statistical analysis confirms a significant difference between the three age groups (median test $p << 0.01$) and the presence of a correlation between β-HCH concentrations and age (Spearman $\rho = 0.55$, $p << 0.01$).

The results of the non-parametric tests show that there are no significant differences in terms of geographical origin among the entire dataset, and therefore data subgroups can be discussed as belonging to a unique population representative of Italian males and females of age 20-65 years.

An overview of β-HCH human biomonitoring data available for other European and non-European countries is presented in *Table 2*. Data appear to be quite variable across countries, and reflect non homogeneous exposure scenarios determined by different situations of past and recent manufacture, and use, of technical HCH.

Eastern Europe countries where technical HCH was manufactured, or where production and application were banned later than 1978, show β-HCH levels considerably higher than those found in western Europe. The highest observed values refer to Kazakhstan, Ukraine, and Romania. According to some authors [21, 22], these concentrations have been determined by an excessive and/or illegal use of technical HCH, and by the presence of large stockpiles of obsolete industrial products, including HCH, in the former Soviet Union and other Eastern Europe countries. In Western Europe countries, studies carried out on samples of the general population in the years 1996-2009 report β-HCH concentrations generally below 50 ng/g, on a lipid base, with the unique exception of Portugal [23, 24].

On the whole, a continuous and time-dependent decline in exposure to β-HCH, as well as to the other HCH isomers, is shown by a number of investigations carried out on human milk in different areas of Europe [25]. Results of the analysis of some thousands of individual human milk samples from Western Germany indicate that, from 1984 to 2001, the levels of β-HCH declined by about 85%, dropping from about 130 to 20 ng/g fat. The same trend is also observed in other European countries, such as Sweden, Norway and UK. Analysis of pooled human milk samples from Bulgaria, Czech Republic, Germany, Ireland, Italy, Luxembourg, Norway, Russia, Spain and Ukraine carried out by WHO [25] confirms this declining trend for most of these Countries.

β-HCH serum concentrations observed in this study fall within the range of values generally observed in western Europe Countries. A comparison of present data with concentrations detected in human milk samples collected in 1987 [18] suggests, also for Italy, a time-dependent decline in exposure, in agreement with what observed for the rest of Europe.

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