Bull. Environ. Contam. Toxicol. (1993) 51:827–831
© 1993 Springer-Verlag New York Inc.

Environmental Contamination and Toxicology

Hexachlorobenzene (HCB) in Human Milk in Spain from 1984 to 1991

C. Conde,[1] C. Maluenda,[2] and C. Arrabal[1]

[1]Servicio de Neonatología and [2]Departamento de Pediatría, Hospital Universitario de San Carlos, Madrid 28040, Spain

Hexachlorobenzene is one of the most persistent organochlorine contaminants known. It is used in the industrial production of pentachlorophenol and is formed as a byproduct in many industrial processes. HCB may be formed during chlorination of waste waters.

As far as 17 years ago (Villar et al, 1976), a mean HCB level of 3.5 ppm was reported in the milk of donors living in a cerealist, nonindustrialized area of Spain. Their figures were similar to those which had been reported in Germany by Rappl et al (1975) at that time. No other report was published in Spain until 1986 when To Figueras et al (1986), in a study made on adipose tissue in Barcelona, also found HCB levels exceptionally high. HCB, together with PCBs and p,p´ DDE, makes up the three major organochlorine contaminants of human milk in Spain and in other countries of South and Central Europe.

The present study covers a period of seven years and includes groups belonging to areas with different environmental conditions all over Spain.

MATERIAL AND METHODS

Material and methods were essentially the same as those described in the first part of this report, except for the clean up of the samples collected between 1984 and 1987. Extracts containing 50mg of milk fat in hexane were chromatographed on a 3g florisil column using hexane-dichloromethane (4+1 v/v) as eluting solvent. A 50ml volume was collected. The eluate was concentrated to 5ml and I.S. (heptachlor) was added. A volume of 200µl (equivalent to 2.5mg of milk fat) was taken and the mixture was carefully concentrated to a final volume of 50µl. The injection volume was 1µl. For the GC MS identification, a multiple ion detection analysis was performed monitoring the sample for ions m/z 284 and 286.

Send reprint requests to C.Conde to the above address

Table 1.HCB in Spanish Human Milk (μg/g fat milk) from 1984 to 1987.

			Nº	HCB (ppm)
Madrid. Urban area	1984	3rd-4th day	25	0.99±0.72
		3rd week	20	1.10±0.65
	1987	3rd week	18	1.12±0.72
Valencia	1986	3rd week	45	0.92±0.52
Industrial areas	1987	3rd-4th day	37	2.91±1.73
Pamplona	1987		46	1.71±0.90
SEG	1984		11	4.99±1.41
	1987		12	3.74±1.93
AR	1987		13	2.27±1.26
PB	1987		13	1.33±0.50

RESULTS AND DISCUSSION

Between January 1984 and November 1987, 204 samples of human milk were obtained from different areas throughout Spain. The collection of samples and information requested from the donors were similar to those reported in the first part of this study. Depending on local conditions, samples were collected on the third - fourth day or in the third week post partum. For comparative purposes, both types of samples were collected in Madrid.

The combined results, expressed on a fat basis, are summarized in Table 1.

HCB residues were present in all the samples analyzed and it could be observed that there was a wide individual variation in the levels measured. Madrid was the most extensively studied community, with 63 samples belonging to its urban area. The average level of the three different groups (3rd - 4th day, third week of 1984 and third week of 1987) were basically similar (about 1 ppm). Comparable values were found in Valencia, in an agricultural area with a cooperative system and well regulated methods of production. A higher average level was found in the industrial areas (2.9ppm). An intermediate value (1.7 ppm) was obtained in Pamplona in samples from several areas of this predominantly agricultural province. The rest of the samples were collected in three small rural communities located far from the industrial areas of the country. SEG, a village whose river had undergone successive processes of chlorination and that had a history of small textile industries that had closed a few years before the samples were obtained, and with a noncontrolled cereal agriculture, showed an average level of 4.99 ppm in 1984. This is the highest level reported in this study. This value declined to 3.74 ppm three years later. AR, another village with a cereal agriculture, showed an average level of 2.27 ppm, comparable to that found in industrial areas, whereas the mean level for PB (1.33 ppm), with a

Table 2.HCB in Spanish Human Milk (μg/g fat milk) from 1990 to 1991.

	Nº	HCB (ppm)
Madrid. Urban area 1991	52	1.04±0.42
1990-1991		
Rural areas in cereal producing provinces	84	0.87±0.37
Urban areas in cereal producing provinces	39	0.98±0.35
Rural areas in irrigation farming provinces	54	0.90±0.31
Urban areas in irrigation farming provinces	38	1.24±0.67
Industrial areas	52	1.74±0.63
Plastic manufacturing area	10	0.92±0.31
Coastal fishing areas	29	1.42±0.58

vegetable agriculture and a forest used for timber exploitation, was similar to that found in Valencia. There were significant statistical differences between the mean values of Valencia and the industrial areas ($p<0.001$) and between Valencia and Pamplona ($p<0.001$). No statistical evaluation was attempted for the three rural zones due to the few samples studied.

A second survey was carried out from January 1990 to October 1991. The results are summarized in Table 2. The 52 donors from Madrid belonged to a population similar to that studied from 1984 to 1987. Regarding the agricultural zones, those described as rural and urban areas in cereal producing provinces were geographically and administratively separated from those studied as SEG and AR in the first report, but there were no remarkable environmental differences between them. Both in 1987 (Table 1) and in 1991 (Table 2), the samples from the industrial zones were taken in Barcelona and Bilbao, although from different areas of their industrial peripheries. The samples from fishing zones were collected in several coastal areas near San Sebastian. A group of 10 samples from donors working in the plastic manufacturing industry was also included. Broadly speaking, all the groups in this second survey showed an average HCB level of about 1ppm, except those in the coastal area and the industrial zones with mean values of 1.42 and 1.74 ppm respectively. A significant statistical difference existed between the samples of the industrial zones and those of the rural cereal producing districts ($p<0.001$) and between the samples of the industrial zones of 1987 and 1991 ($p<0.001$).

Villar et al (1976) published a study of organochlorine contaminants in milk from women living in rural and urban zones of Cordoba. The average HCB level in 40 samples was 3.51 ppm. According to this value, which was reported by Jensen (1983), Spain was considered to be one of the most contaminated countries in Europe in relation to HCB. Cordoba is a nonindustrialized province, with a

predominantly cereal agriculture and thus, the apparent explanation for the high level of HCBs could be no other than the indiscriminate use of HCB as pesticide or the contamination of pesticides by HCB. To Figueras et al (1986) reported an average HCB level of 5.55 ppm in adipose tissue from autopsies carried out in 1984 on subjects who lived in the metropolitan area of Barcelona. Three years later, Camp et al (1989) reported a mean HCB value of 2.99 ppm in adipose tissue obtained from rural areas near Barcelona.

Nineteen of the 37 milk samples collected in 1987 (Table 1) and 40 of those collected in 1991 (Table 2) in industrial zones came from a population which was similar to that reported by To Figueras. It is known that the average levels of organochlorine contaminants are higher in adipose tissue than those found in human milk and this seems to be verified if we compare the results found in Barcelona by To Figueras and by Camp with our results along the last eight years. It is clear that the peripheries of the two most industrialized cities of Spain show a high human contamination by HCB.

More remarkable is the high HCB level (4.99 ppm in 1984, 3.74 ppm in 1987) found in the rural community SEG. Whether these figures can be attributed to a lack of control in the use of pesticides or are due to successive chlorinations of its river or to any other reason remains unexplained. The average HCB level of the 84 cases belonging to the rural areas producing cereals was 0.87 ppm, similar to that found in most of the groups included in this second study.

Since the outbreak of Porphyria Turcica in the late 1950s, the porphyrogenic effect of HCB on humans and animals has been well known. It is said that the HCBs can make a latent congenital pophyria evident. Enriquez de Salamanca et al (1991) point out the remarkable coincidence of higher HCB levels in human milk in Czechoslovakia, Germany and Spain, all three countries having a relatively higher occurrence of Porphyria Cutanea Tarda (PCT). In a study of the frequency of PCT in Madrid between 1965 and 1989, the authors reported the highest incidence between 1977 and 1984, with 60-70 cases per year, and a sharp decrease to less than 25 cases in 1984. This decrease runs parallel to that reported in the HCB levels in adipose tissue and human milk for the same period of time.

HCB seems to have been introduced into Spain during the decade of the seventies. As for its prior or current use, no official information is available. Therefore, it has been impossible to assess if the decline of milk HCB levels in the industrial and cereal producing areas can

be attributed to stricter regulations in the use of HCBs or to a more controlled disposal of industrial wastes.

The fact remains that the overall mean of human milk HCB in Spain is, by far, higher than that reported in the mideighties in the Scandinavian countries (Noren 1983 ; Wickstrom et al 1983), with average levels below 0.1 ppm, and other European countries (Rogirst et al 1983 ; Collins et al 1982) with levels below 0.5 ppm. The highest acceptable level of 0.5 ppm is surpassed by a large number of milk samples in Spain.

Acnowledgments. This work has been supported by a grant from FIIS (0033/89).

REFERENCES

Camps M, Planas J, Gomez Catalan J, Sabroso M, To Figueras J, Corbella J (1989) Organochlorine residues in human adipose tissue in Spain: Study of an agrarian area. Bull Environ Contam Toxicol 42: 195-201

Collins GB, Holmes DC, Hoodles RA (1982) Organochlorine pesticides residues in human milk in Great Britain 1979-1980. Human Toxicol 1: 425-431

Enriquez de Salamanca (1990) Is hexachlorobenzene human overload related to porphyria cutanea tarda? A speculative hypothesis. Med Hypotheses 33: 69-71

Jensen AA (1983) Chemical contaminants in human milk. In:Gunther FA and Gunther JD (ed) Residue Reviews, vol 89. Springer Verlag New York p 76-81

Noren K (1983) Organochlorine contaminants in Swedish human milk from the Stockholm region. Acta Paediatr Scand 72: 259-264

Rappl A, Waiblinger W (1975) Zur contamination von muttermilch mit Rückständem chlorierter kohlenwasserstoffe. Dtsch Med Wschr 100: 228-238

Rogirst A, Vandezande A, Gordst L, Beernaert H (1983) Résidus de pesticides organochlorés et de PCB dans la lait maternel. Archives Belges 41: 424-432

To Figueras J, Rodamilans M, Gomez J, Corbella J (1986) Hexachlorobenzene proceedings of an International Symposium (IARC Scientific Publications nº 77) Morris CR Cabral JRP eds Lyon International Agency for Research of Cancer

Villar LM, Marteache A, Pozo R, Jodral M, Iglesias J (1976) Hexachlorobenceno (HCB) en leche humana en España. Rev Española Pediat 35: 271-274

Wickstrom K, Pyysalo H, Siimes M (1983) Levels of chlordane,hexachlorobenzene,PCB and DDT compounds in Finnish human milk in 1982. Bull Environ Contam Toxicol 31: 251-256

Received January 15, 1993; accepted May 1, 1993.