Bull. Environ. Contam. Toxicol. (1994) 53:877–882

Environmental Contamination and Toxicology

Residual Content of Hexachlorobenzene in Spanish Cheeses

A. Bentabol,[1] M. D. Garrido,[2] M. Jodral[1]

[1]Department of Food Science and Technology, Faculty of Veterinary Medicine, University of Cordoba, Av. Medina-Azahara 9, 14005, Cordoba, Spain
[2]Department of Food Technology, Faculty of Veterinary Medicine, University of Murcia, Campus Espinardo, 30071 Murcia, Spain

Received: 31 December 1993/Accepted: 1 May 1994

Hexachlorobenzene is a fungicide frequently used for the treatment of seeds, specially for cereals against *Tilletia foetidae* and *Tilletia caries*, as well as being part of commercial forms of other fungicides and herbicides, as a contaminant (WHO, 1974).

HCB is a compound with a recognized toxicity, presenting a DL_{50}= 10,000 (EPA, 1984) when administered orally to rats and having produced cases of massive poisoning caused by the diversion for human consumption of seeds treated with this preparation. Besides acute effects, long-term pathogenic effects have been described and according to the International Agencie for Research on Cancer (1978), sufficient evidence of carcinogenicity for HCB has been found in test animals. These effects could have more relevance than the acute ones, since HCB is generally present at residual levels. In this sense, FAO/WHO withdrew the previously allocated conditional ADI in 1978 because the toxicity of HCB is such that contamination of food should be kept as low as possible.

The presence of HCB in milk and dairy products has been reported recently by different authors in Spain (Pozo et al., 1985, Barceló and García, 1987, Garrido, 1990, De la Riva and Anadon, 1991) and in other countries (Frank et al., 1985, Fytianos et al., 1985, Dogheim et al., 1988, Pinto et al., 1990).

The aim of this study was to find out the present levels of HCB in cheeses produced in Spain and to evaluate their possible repercussions on human health.

MATERIALS AND METHODS

146 samples of Spanish cheeses were investigated, 64 of which corresponded to industrially-made cheeses, and the remainder, n = 82, were farm-produced cheeses included in the Catalogue of Spanish cheeses

Correspondence to: A. Bentabol

(Spanish Ministry of Agriculture, Food and Fisheries, 1990). The samples were acquired in ordinary commercial outlets, and kept at 4 °C up to analysis or frozen at -20 °C when it was not possible to analyze them immediately. Fat of the cheese was extracted according to POZO et al. (1985). 45 g of cheese were mixed with previously purified anhydrous Na_2SO_4, homogenized with acetone and petroleum ether, transferred to a separation funnel and liquid-liquid partitioned with bidistilled water. The organic layer was evaporated in a vacuum evaporator, and the fat was kept at -20 °C in glass vials up to analysis. All the reagents were analytical grade and suitable for residue analysis. The glassware was cleaned and prepared according to GARRIDO (1990). Simultaneously, the percentage of fat in the samples was determined using specific butirometers for cheese (DR N. GERBER), previously validated by gravimetrics methods. For the extraction and purification of the HCB residues, a process of hydrolysis with H_2SO_4 was used, which permitted the treatment of larger volumes of fat than other methods and therefore to increase the detection limit of the technique. 2 g of fat were dissolved in 20 ml of n-hexane in a 25 ml screw-tapped glass tube. Then, 1 ml of H_2SO_4 was added, the tube was closed and shaken in an orbital mixer for 30 sec. Subsequently centrifuged at 3,000 rpm for 10 minutes in a labofuge A (HERAEUS) centrifuge. 10 ml of the supernatant was taken and placed in a gauged glass vial for its concentration to 2.5 ml in a sand bath at 45 °C.

A HEWLETT-PACKARD gas chromatograph, mod. 5890, equipped with a ^{63}Ni ECD and a glass column 2 m x 2 mm internal diameter packed with 5 % QF-1 on Chromosorb W-AW 80-100 mesh, was used. The experimental conditions programmed were 225 °C in the injector and detector, and 190 °C in the oven. The carrier gas (Argon:methane, 95:5) flow was 40 ml/min. 5 µl of each of the extracts were injected in the chromatographic system. The quantification was made by the external standard method, using a standard (SUPELCO INC.). Confirmation was carried out using another column with the same physical characteristics as the one used in the analysis but packed with 3 % SE-30.

The recovery of the method was determined by fortification of cheese with known concentration of HCB (2 ng/g of cheese). The cheese was subjected to fat extraction, acid hydrolysis and chromatographic analysis, these processes being carried out in quadruplicate. The mean recovery was 96.9 ± 9.5 % (CV%=9.8).

For the establishment of the detection limit we followed the least amount detectable method (CASTRO et al., 1989), obtaining a detection limit of 0.5 ng/g of fat, and considering 1 ng/g as the quantification limit, because it was the lowest level for which the linearity of the system was shown.

For the evaluation of the possible diferences in the concentrations detected in the two groups of cheeses studied, a single analysis of variance was used, and later a Scheffé test.

RESULTS AND DISCUSSION

The results expressed on fat basis are presented in Table 1. The means and the ranges together with the number of positive samples are shown.

All the samples of Spanish cheeses contained HCB residues, with a mean of 9.26 ng/g fat basis, the maximum level detected was 36 ng/g. Of the two batches of cheeses tested, the industrial ones showed significantly ($p < 0.001$) higher levels of HCB than the farm-produced cheeses. This fact could be due to the mixing of milk used for the making of cheeses in the large dairy industries, where considerable amounts of milk from different sources are used.

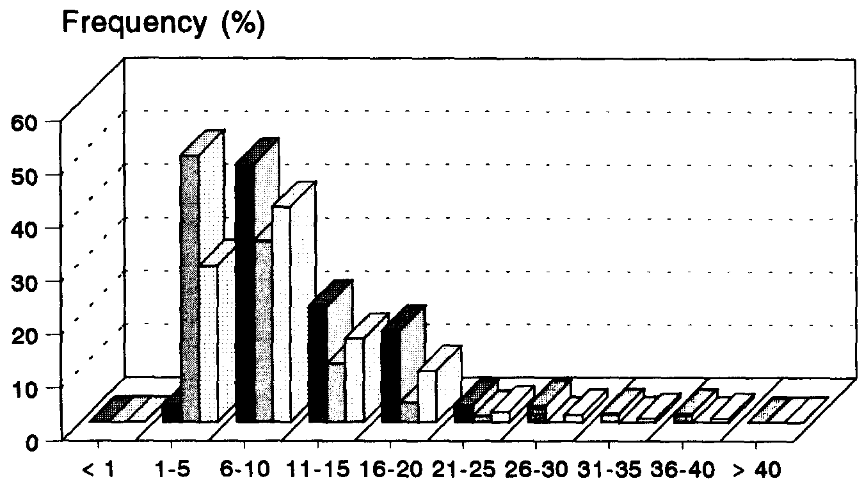
Table 1. Concentration of HCB in groups of Spanish cheeses

	Number of positives	ng/g fat basis Mean	(Range)
Industrially-made cheeses (n=64)	64	12.48	(4 - 36)
Farm-produced cheeses (n=82)	82	6.76	(1 - 24)
Total (n=146)	146	9.26	(1 - 36)

Fig. 1 shows the frequency distribution of the levels of HCB detected, the majority of the samples had concentrations ranging from 1 to 10 ng/g.

The levels detected signify a decrease with respect to those found in investigations made recently in Spain. Pozo et al. (1985) carried out similar research on cheeses in Spain and drop of 94.4 % in the mean HCB concentrations in the time separating both studies has occurred. Garrido (1990) detected 19 ng/g as a mean of HCB on a fat basis in samples of sterilized Spanish milk. These results signify almost double the levels found by us, and this coincides with the mean life of this fungicide reported by Frank et al. (1985). These authors demonstrated that once the use of HCB in agronomic purposes ceased, it disappeared from the environment at a rate of 50 % every 3 years.

In Spain and the rest of the EC the maximum residue limit (MRL) for HCB in this type of food is fixed at 0.25 µg/g on fat basis (Directive EC 86/363, 1986), and this value was not exceeded in any of the 146 samples of



	< 1	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	> 40
I ■	0	3.1	48.4	21.9	17.2	3.1	3.1	1.6	1.6	0
F ▨	0	50	34.1	11	3.7	1.2	0	0	0	0
T □	0	29.4	40.4	15.7	9.6	2	1.4	0.7	0.7	0

Concentration (ng/g)

Figure 1. Frequency distribution of HCB levels detected in Spanish cheeses. (I = industrial cheeses; F = farm-produced cheeses; T = total)

cheese analyzed. Furthermore, the amounts detected signify in all the cases less than the 0.01 % of the ADI recommended until 1978 by the FAO/WHO (1992) for this compound which is 0.6 µg/Kg live weight. For the estimation of the dairy intake a mean weight of 70 kg, a mean percentage of 33.7 of fat in cheese and a mean consumption of cheese of 5.23 kg/person per year were considered (Spanish Ministry of Agriculture, Food and Fisheries, 1989).

The HCB levels detected do not seem to imply any direct risk to the consumer's health, although long-term repercussions of a continued consumption of small amounts of HCB are not fully known. This fungicide is considered as possible carcinogen, so FAO/WHO recomends that HCB levels in food must be the reduced as lowest as possible.

It can be seen how, in comparison with the results of previous research, we find ourselves in the process of a gradual decrease in the levels of HCB in the environment which can be extended to dairy products, as a result of the prohibition of the use of this preparation for agricultural purposes.

It is to be pointed out, however, that although we observed lower levels than those found by Spanish researchers in the 70's and the 80's, the fact that there were residues of this fungicide in all the samples analyzed indicated a high degree of its dispersion in the environment. An increase in the percentage of residue detection was observed with respect to the experiments of Pozo et al. (1985) and Garrido (1990), this authors detected HCB in the 68.9 % and 57,7 % of the cases, respectively. This could be due to an improvement in the sensitivity of our method with respect to those employed by other authors, and to the appearance of HCB as an impurity of the fungicide Pentachloronitrobenzene (PCNB) (Pozo et al., 1985), as well as an industrial contaminant, since Hexachlorobenzene is often used in the chemical industry as a base for the manufacture of other compounds. In the opinion of Frank et al. (1985) the industrial use of HCB might be the fundamental cause of environmental contamination at the present time. HCB could also be present in food as a result of its use as a fungicide, in spite of its prohibition in Spain.

We consider that it is necessary to control the sources of environmental contamination by HCB since although only low concentrations of this organochlorine compound were found in the samples of cheese tested, this contaminant has been seen to be highly dispersed in the environment.

Acknowledgments. The authors wish to thank Ms. Gloria Fernández-Marín for her invaluable collaboration and analytical assistance during the development of this work. This research is supported by the project ALI 92/0991 of the Interministerial Commission of Science and Technology of Spain.

REFERENCES

Barcelo D, Garcia-Puignou L (1987) Residuos de plaguicidas en leches U.H.T. españolas determinados por cromatografía de gases de alta resolución. Rev. Agroquím. Tecnol. Aliment. 27:583-589

Castro M, Gascon S, Pujol M, Sans JM, Vicente L (1989) Validación de Métodos Analíticos. Monografía de la Asociación Española de Farmacéuticos de la Industria, Sección Catalana, Comisión de normas de buenas prácticas de fabricación y control de calidad, Barcelona

De la Riva C, Anadon A (1991) Organochlorine Pesticides in Cow's Milk from Agricultural Region in Northwestern Spain. Bull. Environ. Contam. Toxicol. 46:527-533

Directive of the Council of the European Communities 86/363/CEE (1986) Fijación de contenidos máximos para los residuos de plaguicidas sobre y en los productos alimenticios de origen animal. Bol. Of. de las Comunidades Europeas 221:43-47

Dogheim SM, Almaz MM, Kostandi SN, Hegazy ME (1988) Pesticide Residue in Milk and Fish Samples Collected from Upper Egypt. J. Assoc. Off. Anal. Chem. 71:872-874

FAO (1978) Pesticide residues in food-1978. Evaluation 1978, 15 sup, Food and Agriculture Organization of the United Nations, Rome

Frank R, Braun HE, Sirons GH, Rasper J, Ward GG (1985)

Organochlorine and Organophosphorus Insecticides and Industrial Pollutants in the Milk Supplies of Ontario-1983. J. Assoc. Off. Anal. Chem. 48:499-504

FYTIANOS K, VASILIKIOTIS G, WEIL L, KAVLENDIS E LASKARIDIS N (1985) Preliminary Study of Organochlorine Compounds in Milk Products, Human Milk, and Vegetables. Bull. Environ. Contam. Toxicol. 34:504-508

GARRIDO MªD (1990) Niveles de pesticidas organoclorados y bifenilos policlorados en leches esterilizadas españolas.Y estudio experimental del efecto de la temperatura sobre estos compuestos. Doctoral Thesis. Faculty of Veterinary. University of Córdoba.

INTERNATIONAL AGENCIE FOR RESEARCH ON CANCER (IARC) (1988) Overall evaluations of carcinogenicity: an updating of IARC monographs, 1-42. International Center for Cancer Research, Lyon

PINTO M, MONTES L, ANRIQUE R, CARRILLO R, TAMAYO R, CRISTI R (1990) Residuos de plaguicidas organoclorados en leche de vaca y su relación con alimentos para uso animal como fuentes de contaminación. Arch. Med. Vet. de Chile 22:143-153

POZO R, POLO L, JODRAL M, JORDANO R, ZURERA G, RINCON F (1985) Hexaclorobenceno en quesos españoles. Rev. de Toxicología 2:198-205

SPANISH MINISTRY OF AGRICULTURE, FOOD AND FISHERIES (1989) El consumo alimentario en España. Ministerio de Agricultura, Pesca y Alimentación, Secretaría General Técnica, Madrid

SPANISH MINISTRY OF AGRICULTURE, FOOD AND FISHERIES (1990) Catálogo de quesos de España. Ministerio de Agricultura, Pesca y Alimentación, Secretaría General Técnica, Madrid

US EPA (1984) Analytical Reference Standards and supplemental data: The pesticides and Industrial Chemical Repository. EPA-600/4-84-082. U.S. Environmental Protection Agency, Las Vegas

WHO (1974) Evaluation of some pesticides residues in food. WHO Pesticide Residues Series 4. Geneva

WHO (1992) Consecuencias sanitarias del empleo de plaguicidas en la agricultura. World Health Organization, Geneva