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Original article

Organochlorine pesticide residues in Galician (NW Spain) honeys

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Summary — Organochlorine pesticide residues were analyzed by capillary gas chromatography-electron capture detection (GC-ECD) on 101 Galician (NW Spain) honey samples. Of the 101 honey samples, 13 were free from detectable residues. The pesticides found were HCH (isomers other than lindane) present in 47 samples (trace to 161 μg/kg), lindane in 57 samples (trace to 59 μg/kg), heptachlor in 29 samples (trace to 57 μg/kg) aldrin in 36 samples (1 to 150 μg/kg), dieldrin in 9 samples (trace to 13 μg/kg), endrin in 1 sample (7 μg/kg), op'DDT in 7 samples (1 to 12 μg/kg), pp'DDT in 18 samples (1 to 61 μg/kg) and methoxychlor in 11 samples (19 to 593 μg/kg).

honey / organochlorine pesticide / residue / Spain

INTRODUCTION

Although the introduction of DDT and other organochlorine pesticides was beneficial originally for both farming and public health, the massive use of these products has proved undesirable. Their chemical stability and liposolubility result in accumulation in both the environment and animal tissue and, hence, in serious problems of toxicity.

The chronic toxicity associated with continual ingestion of small doses over long periods includes teratogenic, carcinogenic, oestrogen-inducing, hepatotoxic, immunosuppressive and neurotoxic effects. Acute toxic effects, which can be caused not only by the ingestion of relatively large doses but also by the mobilization of lipids during strenuous exercise or stress, include neuronal alterations, irritation of skin and mucosa, tremor, convulsions, and even shock and

death (Horiuchi *et al,* 1978; Nelson *et al,* 1978; Morgan *et al,* 1980; Chambers and Yarbrough, 1982; Concon, 1988). Because of these risks, many countries have now banned or severely limited the use of organochlorine pesticides, and have established legal limits to their concentrations in foodstuffs. The purpose of this work was to investigate organochlorine pesticide residue levels in honey samples from Galicia, a region in NW Spain of approximately 29 434 km^2 in area.

MATERIALS AND METHODS

Samples

This study was done on 101 honey samples harvested in September 1990 from individual apiaries in all 4 Galician provinces (fig 1): La Coruña (31), Pontevedra (18), Orense (24) and Lugo (28).

Methods

Organochlorine pesticide residues were determined according to the method of Fernández

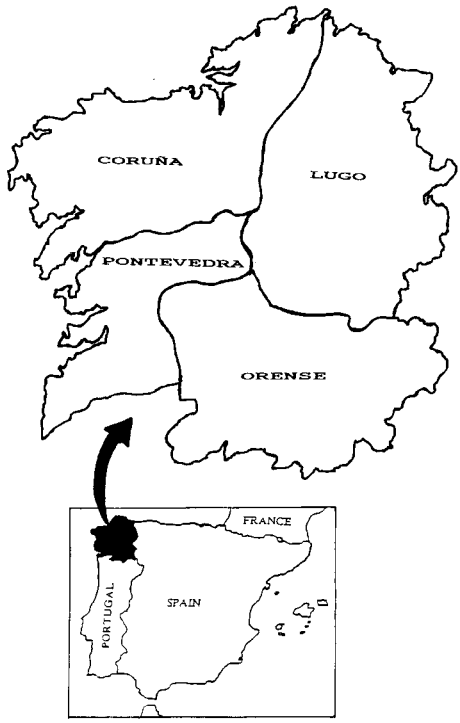

Fig 1. Geographical origin of samples.

Table I. Organochlorine pesticide residue levels found in Galician honeys.

Pesticide	*% of samples with residues*	*Range of amounts (μg/kg)*
HCH	47	trace to 161
Lindane	57	trace to 59
Heptachlor	29	trace to 57
Aldrin	36	1–150
Heptachlor epoxide	0	
Dieldrin	9	trace to 13
Endrin	1	7
op'DDT	7	1–12
pp'DDT	18	1–61
Methoxychlor	11	19–593

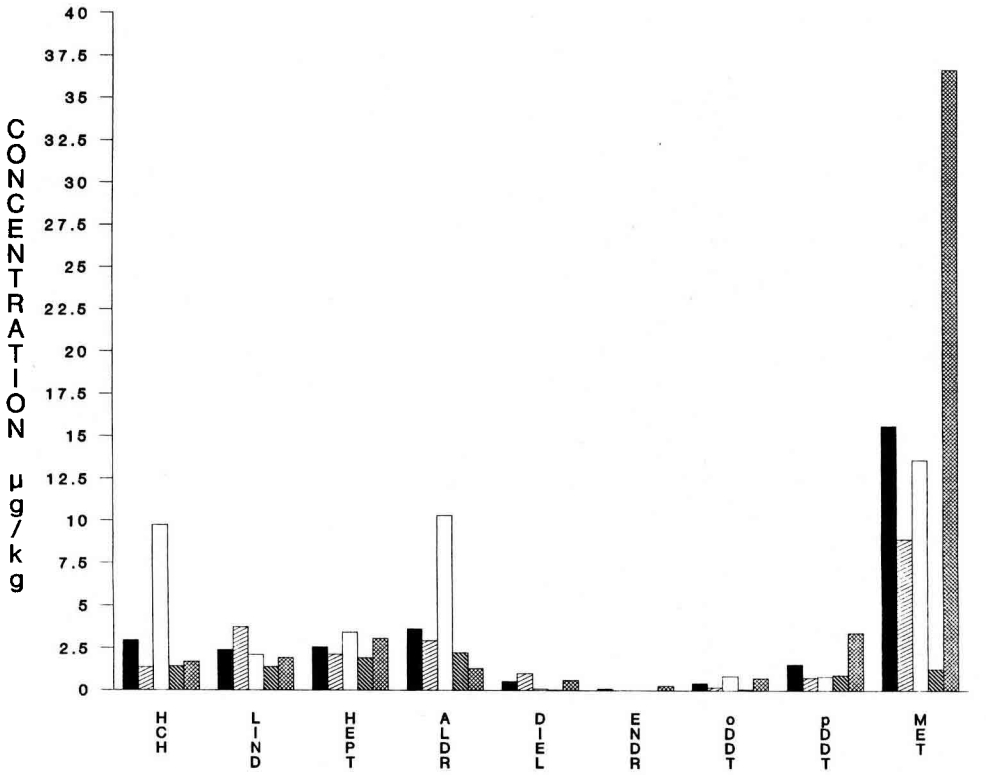

Fig 2. Mean values of organochlorine pesticide residue levels found in Galician honeys and samples from individual provinces. HCH includes isomers other than lindane; LIND = lindane; HEPT = heptachlor; ALDR = aldrin; DIEL = dieldrin; ENDR = endrin; oDDT = op'DDT; pDDT = pp'DDT; MET = methoxychlor. ■ Galicia; ▨ la Coruña; □ Pontevedra; ▧ Orense; ▩ Lugo.

Muiño and Simal Lozano (1991) based on hexane extraction, adsorption chromatography on a Florisil Sep-Pak cartridge and elution with 15% diethyl ether in hexane. Final detection and quantification or organochlorine pesticides were performed by capillary gas chromatography with electron capture detection. The quantification limit obtained for different pesticides ranged from 0.56 to 2.78 µg/kg and recoveries from fortified honey samples averaged 89.6%.

For developing these analysis on the honeys from Galicia, we used a Perkin-Elmer Series 8700 gas chromatograph equipped with a ^{63}Ni electron-capture detector, a split/splitless injector and RSL-200 capillary column (25 m x 0.22 mm id, 0.25 µm film thickness).

RESULTS AND DISCUSSION

Table I shows the percentage of samples with residues and range of amounts for each pesticide studied in the 101 honey samples. Figure 2 shows the mean values obtained for the above pesticides found in the samples analyzed, both in Galicia and in individual provinces. Heptachlor epoxide residues were also analyzed but could not be detected in any sample, despite the detection of heptachlor residues. Most of the detected pesticides have been banned

in Spain and only lindane and methoxychlor are commonly used in agricultural practice.

These compounds are environmental pollutants and, due to their high fat solubility, an accumulation of chlorinated pesticide residues in wax can occur. Foraging honey bees might add new supplies of pesticides by their deposition of loads of nectar, honeydew and pollen in wax cells. This could result in an accumulation of pesticide residues in wax and, therefore, in honey and bee-collected pollen, although earlier studies have reported much lower pesticide levels in apicultural foods than in wax (Gayger and Dustmann, 1985).

The only earlier study carried out in Spain (Serra Bonhevi, 1985) reported very low residue levels in the honey samples analyzed. We think that this could be a dilution effect since the samples studied were collected from very large containers for wholesalers.

The organochlorine pesticide residues found in this study are of the same levels as those reported in earlier studies from other European countries on honey samples (Dzilinski and Szymanowska-Bielawska, 1975; Sabatini and Savigni, 1976; Tsvetkova *et al*, 1981; Bigazzi Grasso and Capei, 1983; Gayger and Dustmann, 1985; Rexillius, 1986). They reported levels ranged from traces to 40 μg/kg, with the exception of the results of Bigazzi Grasso and Capei (1983) for DDT, which ranged between 28.4 and 1 018 μg/kg. Moilanen *et al* (1986) and Orbaek (1987) did not find detectable organochlorine pesticide residue levels.

Current Spanish legislation prohibits the presence of any trace of organochlorine pesticide in honey, but this is an unrealistic goal because hive locations are generally surrounded by agricultural land on which these pesticides were or are still used. It is therefore desirable to follow the same practice as for other foodstuffs by establishing non-zero maximum tolerable limits relating the tolerable daily intake of the pesticides to the level of honey consumption among the population.

Spanish legislation does not explicitly fix limit values for organochlorine pesticides in honeys. We believe that reference to farm produce, for which explicit limit values have been fixed, provides a useful orientation in the interpretation of our results. Legal limits for most organochlorine pesticides in plant produce is legislated to range between 10 and 100 μg/kg, but the limit for methoxychlor is 10 mg/kg. The levels we detected in honey are therefore not alarming; all are well below the above limits, including the concentrations of methoxychlor (19–600 μg/kg).

ACKNOWLEDGMENTS

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Résumé — Résidus de pesticides organochlorés dans les miels de Galice (Espagne). À cause de leur stabilité chimique et de leur caractère liposoluble qui engendrent des problèmes de toxicité, les pesticides organochlorés ont été bannis dans de nombreux pays ou leur utilisation grandement limitée et des limites légales de concentration dans les produits alimentaires ont été définies. Le but de ce travail est de rechercher les résidus de pesticides organochlorés dans des échantillons de miel de Galice, région de 29 434 km^2 située dans le nord-ouest de l'Espagne. L'étude a porté sur 101 échantillons de miel récoltés en septembre 1990 dans des ruchers répartis dans les 4 provinces de Galice (fig 1) : La Coruña (31), Pontevedra (18), Orense (24)

et Lugo (28). Les résidus d'organochlorés ont été déterminés selon la méthode mise au point par Fernández Muiño et Simal Lozano (1991), méthode basée sur l'extraction à l'hexane, la purification par chromatographie d'adsorption sur une cartouche Florisil Sep-Pak et l'élution avec un mélange hexane-diéthyl éther (15%). La détection finale et la quantification des organochlorés a été faite par chromatographie en phase gazeuse avec détection de capture d'électrons. Le tableau I donne le pourcentage d'échantillons présentant des résidus et leurs valeurs extrêmes pour chaque pesticide étudié. La figure 2 donne les valeurs moyennes des pesticides, dont on a retrouvé des résidus, pour chacune des provinces et pour l'ensemble de la Galice. Les résidus retrouvés sont du même ordre de grandeur que ceux mentionnés dans les études antérieures portant sur des miels d'autres pays européens. Dans le cas du miel, la législation espagnole actuelle interdit la présence de traces d'organochlorés quels qu'ils soient, mais c'est un objectif non réaliste car les emplacements de ruches sont généralement situés au milieu de terres agricoles sur lesquelles ces pesticides ont été ou sont encore utilisés. Il est donc souhaitable de suivre l'exemple d'autres produits alimentaires en déterminant des limites admissibles maximum en fonction de la dose journalière admissible de pesticides et de la consommation de miel par la population.

miel / pesticide organochloré / résidu / Espagne

Zusammenfassung — Rückstände von chlorkohlenwasserstoffhaltigen Pestiziden in galicischen Honigen. Chlorkohlenwasserstoffhaltige (CKW) Pestizide verursachen durch ihre chemische Stabilität und ihre Fettlöslichkeit ernsthafte toxische Probleme. Viele Länder haben inzwischen diese Pestizide verboten oder ihre Anwendung deutlich eingeschränkt. Sie haben gesetzliche Obergrenzen für ihre Konzentration in Nahrungsmitteln festgelegt. In dieser Arbeit wurde die Höhe der Rückstände von CKW-haltigen Pestiziden in Honigproben aus Galicien, einem Gebiet von 29,434 km^2 in Nordwest-Spanien, untersucht. Es wurden 101 Honigproben untersucht, die 1990 von verschiedenen Bienenständen in allen 4 galicischen Provinzen (Abb 1) gesammelt wurden: La Coruna (31), Pontevedra (18), Orense (24) und Lugo (28). Die Rückstände wurden mit einer Methode bestimmt, die von Fernandez Muino und Samla Lozano (1991) entwickelt wurde. Sie beruht auf einer Hexanextraktion, mit anschließendem Reinigungsschritt durch Absorptionschromatographie über eine Florisil Sep-Pak Säule und Elution mit 15% Diethylether in Hexan. Die endgültige qualitative und quantitative Analyse erfolgte mit Hilfe der Kapillar-Gaschromatographie (GC) mit einem Elektronenfangdetektor (ECD). Tabelle I zeigt den Prozentsatz der Proben mit Rückständen. Der Mengenbereich wurde für jedes nachgewiesene Pestizid in den 101 Honigproben untersucht. Abbildung 2 zeigt die Mittelwerte der Pestizide, die in den Honigproben gefunden wurden, sowohl für ganz Galicien als auch für seine Provinzen. Die gemessenen Rückstände der CKW-haltigen Pestizide haben die gleiche Höhe wie sie bereits in früheren Untersuchungen in anderen europäischen Ländern beschrieben wurde. Im Fall von Honig ist nach momentanem spanischen Gesetz jeglicher noch so kleiner Rüsckstand von CKW-haltigen Pestiziden verboten. Aber das ist ein unrealistisches Ziel, denn die Bienenvölker befinden sich im allgemeinen innerhalb von landwirtschaftlichen Gebieten, in denen diese Pestizide benutzt wurden oder noch werden. Es wäre daher wünschenswert, hier derselben Praxis für andere Nahrungsmittel zu folgen und eine Obergrenze oberhalb von Null einzuführen. Die maximal tolerierbaren Grenzwerte sollen von der tolerablen täglichen Aufnahme und der

Menge des täglichen Verzehrs von Honig in der Bevölkerung abhängen.

Honig / Pestizid / Rückstand / Chlorkohlenwasserstoffe / Spanien

REFERENCES

Bigazzi Grasso C, Capei R (1983) Organochlorine pesticide residues in honey samples. *Ig Mod* 80, 975-980 (in Italian)

Chambers JE, Yarbrough JD (1982) *Effects of Chronic Exposure to Pesticides on Animal systems*, Raven Press, New York, USA

Concon JM (1988) *Food Toxicology: Contaminants and Additives*. Marcel Dekker Inc, New York, USA

Dzilinski E, Szymanowska-Bielawska K (1975) Level of chlorinated insecticides in nectar honey from the Warsaw area and Warsaw province. *Med Weter* 31, 418-420 (in Polish)

Fernández Muiño MA, Simal Lozano J (1991) Simplified method for the determination of organochlorine pesticides in honey. *Analyst* 116, 269-271

Gayger J von, Dustmann JH (1985) Rückstandsuntersuchungen von Bienenprodukten Wachs, Honig und Pollen. *Arch Lebensmittelhyg* 36, 77-100

Horiuchi N, Ando S, Suzuki A (1978) Photo sensitization due to pesticides. *J Jpn Assoc Rural Med 27*, 450-451

Moilanen R, Kumpulainen J, Pyysalo H (1986) Margarine, butter, honey and vegetable oils as sources of organochlorine compounds in the Finnish diet. *Ann Agric Fenn* 25, 177-185

Morgan DP, Lin LI, Saikali HH (1980) Morbidity and mortality in workers occupationally exposed to pesticides. *Arch Environ Contam Toxicol* 9, 349-382

Nelson JA, Struck RF, James R (1978) Estrogenic activities of chlorinated hydrocarbons. *J Toxicol Environ Health* 4, 325-339

Orbaek K (1987) Pesticide residues in Danish food. Report 1987, OYB-147; Order No PB88-139514, 138 p (in Danish)

Rexillius R (1986) Residues of pesticides in oilseed-rape honey (crop 1984) from Schleswig-Holstein. *Nachrichtenbl Dtsch Pflanzenschutzdienstes (Braunschweig)* 38, 49-56 (in German)

Sabatini AG, Savigni G (1976) Study of residues of chlororganic and phosphorganic phytodrugs in samples of honey produced in Emilia-Romagna. *Riv Sci Tecnol Alimenti Nutr Um* 6, 167-170 (in Italian)

Serra Bonhevi J (1985) Determinación de pesticidas organoclorados en la miel. *Alimentaria* 22, 55-58 (in Spanish)

Tsvetkova TS, Peneva V, Grigorova D (1981) Residual amounts of pesticides in honey. *Vet Med Nauki* 18, 93-98 (in Russian)