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Endosulfan Residues in Brazilian Tomatoes and Their Impact on Public Health and the Environment

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From the organochlorine group of pesticides endosulfan is frequently selected for use as a general purpose insecticide. In Brazil, its application is, by regulation, only permitted on plantations of sugar cane and cacao, with a bulk residue limit of 10 ppb, and coffee, where an upper limit of 40 ppb in the beans has been established.

Little reliable data is available regarding the presence of pesticide residues in locally produced Brazilian foods. A restricted monitoring program, located in the south of this country and around the city of Belo Horizonte, reported endosulfan residues of 4 to 220 ppb in locally grown fruits and vegetables (Soares 1986). More recently a similar study, and again in the south but now centred on the city of So Paulo, broadly confirmed these results (Gebara et al 1995; Barretto et al 1996). Additionally endosulfan contamination has been frequently detected in the commercial production of strawberries; in a study covering the years 1983 to 1988 it was found that 29% of samples were contaminated, see Guindani and Ungaro 1988 and, in the region of Campinas, at the high level of 60 ppb (Oliveira and Toledo 1995).

Endosulfan has been shown to be highly toxic to freshwater fish, promoting metabolic and reproductive disorders (Viana and Martins 1995; Mishra and Shukla 1997). Freshwater prawns are also known to be highly susceptible to this product (Lombardi et al 1997). Additionally the accumulation of endosulfan in marine species has been reported (Naqvi and Vaishnavi 1993). For these reasons due care must be exercised when using this product in close proximity to surface water; the EPA recommends that the level of endosulfan in lakes and rivers should not exceed 74 ppb. Precautions must also be taken to protect workers from exposure to this product. OSHA has set a TWA exposure limit of 0.1 mg per cubic meter of air. Endosulfan may be absorbed via the gastrointestinal tract, by inhalation or by contact with the skin. Contaminated workers are reported to suffer from irritability, convulsions and related neurological disorders (Lemes et al 1993).

In this study a simple multi-residue method was developed for the quantification of endosulfan in tomatoes. Data are reported, using this analytical method, for tomatoes grown intensively in a region located approximately 120 km from Recife, the state capital of Pernambuco, and located in the North-East of Brazil. This study, the first systematic investigation of possible pesticide contamination in

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primary foods in this region of Brazil, is part of a wider program, which also contains a detailed questionnaire relating to utilisation of insecticides (Araújo 1998). The objective is to evaluate the overall impact of pesticides upon public health and the environment

MATERIALS AND METHODS

Twenty two individual tomato plantations, covering an area of 42.6 hectares, which statistically represented a 150 hectare area, were systematically investigated from December of 1996 until April 1997. Three fruits were collected at each of 15 points, for each plantation. An imaginary line connecting these 15 uniformly distributed sampling points traced the letter Z and covered the entire plantation, with the exception of the periphery (Ambrus 1984). Each fruit was wrapped in aluminium foil, placed in an Isopor container filled with ice and transported directly to the laboratory for analysis. At the laboratory tomatoes were quartered and opposite segments discarded, in order to reduce the bulk of the material needing to be processed. Following this step the tomatoes were ground into an homogeneous paste, a small portion of which was used directly for analysis and the remainder frozen for subsequent study.

Additionally, at the time of sample collection, efforts were made to interview each and every cultivator. This action proved difficult to complete in full, due to the wide geographic area covered and the nature of daily rural life and activities in this part of Brazil. Nevertheless, 159 detailed questionnaires were submitted and completed, representing about 30% of the total workforce of this area, in which were recorded: the types and quantities of pesticides used, their mode of application, the age and identification of the workers who applied these products, their current and recent state of health, how these products were obtained, if technical assistance was available for their selection and the ultimate destination of the empty containers.

Pesticide analytical standards were purchased from Ehrenstorfer, and the solvents, iso-octane, ethyl acetate, petroleum ether and methylene chloride, all pesticide grade, were purchased from Merck

Standard stock solutions of α, β and endosulfan sulfate were prepared in iso-octane with the following concentrations 248, 260 and 253 µg/ml. Working standard solutions were prepared by dilution of the above with iso-octane, all solutions being stored at -20° C.

Twenty five millilitres of ethyl acetate were added to 15 grams of a tomato sample and, as and when required, an equal quantity of a spiked sample and a blank solvent sample then vigorously agitated twice and left for 2 minutes. Anhydrous sodium sulfate was added and the mixture again vigorously shaken (Dutch Ministry of Public Health, Welfare and Sport 1996); a 5 ml aliquot of the organic phase was removed and evaporated to dryness using a stream of nitrogen gas.

The dried extract was re-suspended with 5 ml of petroleum ether and transferred to a glass column packed with 6 gram of Florisil, which had previously been de-activated to 4%. Organochlorine residues were eluted from this column using approximately 60 ml of two differing mobile phases, initially a 4:1mixture of

petroleum ether and methylene chloride and subsequently 6:4 of the same solvents, at the flow rate of 5 ml per minute (Mitchell 1976; Luke et al 1975). The two samples obtained above were then concentrated, firstly by evaporation in a rotary evaporator to about 5 ml followed by the removal of the remaining solvent with a controlled stream of nitrogen gas. Residues were then re-suspended in 5 ml of petroleum ether prior to analysis by gas chromatography.

A HP-5890 series II gas chromatograph, equipped with an electron-capture detector and a HP-3396-II integrator were used to perform the analysis. This instrument was fitted with a 5 % methyl-phenyl-silicone capillary column, 25 m (0,2 mm I.D., 0,33 µm). The chromatographic conditions were: an oven temperature programmed from 70 to 180° C at the rate of 250 C/min and the heating rate then reduced to 10° C/min until 230° C, with a 30 min hold at this temperature. The injection port set to 220° C and the detector to 280° C with 1 ml/min of Helium as the carrier gas, and 1 µl samples directly injected.

Limits of detection (LOD) and quantification (LOQ) were established by the addition of known amounts of α, β and endosulfan sulphate to 15 g of tomato samples. These samples were subjected to the same preparative procedures as described above, and the chromatographic analysis repeated 5 times.

The recovery coefficient (%) was obtained by the addition of endosulfan, with calculated concentrations in the range 4 to 400 ppb, to 15 g of samples which were then analysed as described in sample processing. These measurements were made 4 times.

The selectivity of the method was investigated by examining the separation of α, β and endosulfan sulfate peaks from those of the other constituents of tomatoes, these fruits being selected from plantations known not to employ insecticides.

Calibration curves for α, β and endosulfan sulfate were obtained for the ranges 2.5 to 14.9, 2.6 to 15.6 and 5.1 to 25.3 ng/ml, respectively, and their linearity was evaluated by the r^2 test. These determinations were replicated 5 times. In a tomato sample, the final result was expressed as the sum of α, β and endosulfan sulfate concentrations.

RESULTS AND DISCUSSION

The technique presented for the extraction and purification of endosulfan from tomatoes is both simple and rapid, and may be applied to other groups of insecticides. The results obtained from the validation tests, see Table 1 and Figure 1, are acceptable. The limit of quantification (LOQ) was 4.0 ppb for α and β endosulfan and 6.0 ppb for endosulfan sulfate, as determined by the level at which the variance coefficient was less than 20%.

From Table 2 it is seen that 28% of the samples collected tested positive for endosulfan although only one cultivator admitted to using this product. Their omissions may be judged accidental, since endosulfan is not authorized for use with tomatoes in Brazil, or may simply reflect their lack of basic education and technical support in the use of insecticides.

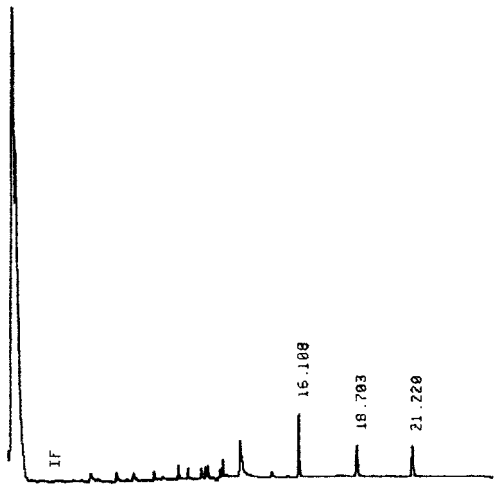

Figure 1. Chromatogram of a spiked endosulfan (α, β and sulfate) tomato sample, with concentrations varying between 4,0 and 6,1 ppb and its retention times in minutes.

Table 1. Numerical values of validation procedures for α, β and endosulfan sulfate

parameter	α– endosulfan	β– endosulfan	endosulfan sulfate
Concentration range (ng/ml)	2,5 – 14,9	2,6 – 15,6	5,1 – 25,3
Recovery (%)	86,3	81,4	74,1
LOD (ppb)	1,9	2,1	4,7
LOQ (ppb)	4,0	4,2	6,1
r^2	0,998	0,998	0,994

LOD: limit of detection
LOQ: limit of quantification

Examination of the questionnaires revealed a wide variation in both the type, frequency and quantity of insecticide applied. There was also little evidence of the knowledge of the possible increased resistance of pests to these products due to their over utilisation. It may be also noted that 5 other prohibited products were mentioned, three organochlorines and two of unknown origin and composition. Additionally, knowledge of the possible deleterious effects upon an individual health, or the environment, by the incautious use of insecticides was also lacking. Several cultivators reported symptoms of skin diseases (36,4%), nausea and modifications to sleep patterns (32,5%) and fetus abortion (70,4%). This region has no public health workers specifically trained in assisting, or educating, workers exposed to possible contamination by insecticides. Eighteen and one half percent of empty, or nearly empty, insecticide containers were often discarded haphazardly within the plantations where rainwater would certainly wash any residues into local water sources.

Endosulfan contamination has been widely detected in tomatoes grown for the table market, the highest level being 510 ppb.

Table 2. Identification of cultivators, farming area, sampling date and total endosulfan residues in the 32 tomato samples from the studied area

number	cultivator	farming area	sampling date	endosulfan (µg/kg)
1	S.L.N.	1,5 ha	12/03/96	< LOQ
2	S.J.S.	3,0 ha	12/03/96	< LOQ
3	R.S.S.	0,5 ha	12/03/96	280
4	G.S.S.	2,0 ha	12/03/96	< LOQ
5	N.B.S.	1,0 ha	01/08/97	< LOQ
6	M.G.	2,0 ha	01/08/97	< LOQ
7	J.A.	2,5 ha	01/08/97	390
8	J.A.S.	2,0 ha	01/08/97	< LOQ
9	S.F.C.	1,0 ha	02/05/97	510
10	F.M.S.	1,5 ha	02/27/97	< LOQ
11	J.S.S.	1,0 ha	02/27/97	30
12	F.V.S.	1,5 ha	02/27/97	40
13	A.G.	2,0 ha	02/27/97	< LOQ
14	M.F.C.	2,0 ha	02/27/97	35
15	R.O.	0,5 ha	03/12/97	< LOQ
16	J.A.M.	1,0 ha	03/12/97	< LOQ
17	G.	0,5 ha	03/12/97	130
18	S.V.	1,0 ha	03/12/97	102
19	F.M.	2,0 ha	03/12/97	200
20	J.E.M.	2,0 ha	03/19/97	< LOQ
21	F.V.S.	1,0 ha	03/19/97	< LOQ
22	L.N.	1,0 ha	03/19/97	< LOQ
23	F.	1,0 ha	03/19/97	< LOQ
24	M.P.S.	1,0 ha	19/03/97	< LOQ
25	J.G.S.	2,0 ha	26/03/97	< LOQ
26	E.S.S.	2,0 ha	26/03/97	< LOQ
27	M.B.S.	0,5 ha	08/04/97	< LOQ
28	J.J.S.	0,4 ha	08/04/97	< LOQ
29	M.J.L.	1,5 ha	08/04/97	< LOQ
30	S.M.L.	0,5 ha	08/04/97	< LOQ
31	J.M.S.	0,5 ha	08/04/97	< LOQ
32	M.J.A.	0,7 ha	08/04/97	< LOQ

LOQ: limit of quantification

In Brazil although stringent regulations exist for the use of insecticides few states effectively enforce these controls. The negative effects of the indiscriminate use of these products are seen to be not sufficiently explained and demonstrated to the local labour force, clearly there is evidence of a significant lack of public education and awareness.

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