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^{14}C-Chlorpyrifos Residues in Tomatoes and Tomato Products

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Chlorpyrifos (*o, o,* -diethyl *o*-3, 5, 6-trichloro-2-pyridyl phosphoro thioate) is a broad spectrum insecticides effective by contact, ingestion and vapour action to control *Coleoptera, Diptera, Homoptera* and *Lepidoptera* in soil and foliage of citrus, coffee, cotton, maize, sugar beet and vegetable crops (Worthing and Hance 1991). This organophosphorus insecticide is an anticholinesterase agent with moderate toxicity: acute oral (rat) LD_{50} = 82-155 mg/kg, dermal LD_{50} = 202 mg/kg. Previous investigations showed that the compound did not penetrate either roots or leaves, but only metabolites entered the plant and the amount of radioactive compound found inside the plant was 0.5 to 2 % of the applied dose (Smith et al. 1967). The rate of dissipation depends on the nature of insecticide and the influence of environmental factors as well as the plant structure (Lee and Cheng 1983).

In Turkey, tomatoes are ranked fourth in production after wheat, sugar beet and barley. However tomato plants are infested by many different insects such as *Heliotis armigera* Hbn, *Aphididae, Agrotis* spp., *Gryllotalpa gryllotalpa* during growing period. Use of chlorpyrifos in vegetable cultivation was about 35000 kg a.i. per year (Anonymous 1996). It is reported that maximum residue level (MRL) for chlorpyrifos is 0.5 mg/kg and acceptable daily intake (ADI) is 0.01 mg per kg body weight in human.

The purpose of this study was to determine the level and nature of ^{14}C-chlorpyrifos residues in tomatoes and tomato products and to investigate the effect of food processing on chlorpyrifos residue by radiotracer technique.

MATERIALS AND METHODS

^{14}C-chlorpyrifos (*o, o,* -diethyl *o*-3, 5,6-trichloro-2-pyridyl phosphoro thioate) labelled at positions 2 and 6 of the pyridine ring was obtained from IAEA. The chemical had a specific activity of 1.09 MBq/mg and radiochemical purity was over 95%. Commercial chlorpyrifos was supplied by Dow-Elanco. Scintillation cocktails were *Insta Gel* (Packard, 6013004) and *Rotiszint eco plus* (Carl Roth GmbH+Co., Karlsruhe). Merck Kieselgel 60 F-254 (Art. 5715) plates were used for thin layer chromatography.

Tomato plants of Super California variety were grown in boxes dimensions of 60x60x60 cm constructed from galvanized steel under outdoor conditions. The base of the boxes contained holes to permit the drainage of excess water (Kohli et al. 1973). Soil texture was sandy clay.

^{14}C-chlorpyrifos was diluted with nonlabelled chlorpyrifos and then applied to the leaves until runoff point with a sprayer one month after transplanting of seedlings to the boxes. Plants were treated twice during growing season. Specific activity of the applications was 2510 dpm/µg (334.5 kBq ^{14}C-chlorpyrifos + 7.8 mg cold chlorpyrifos per plant).

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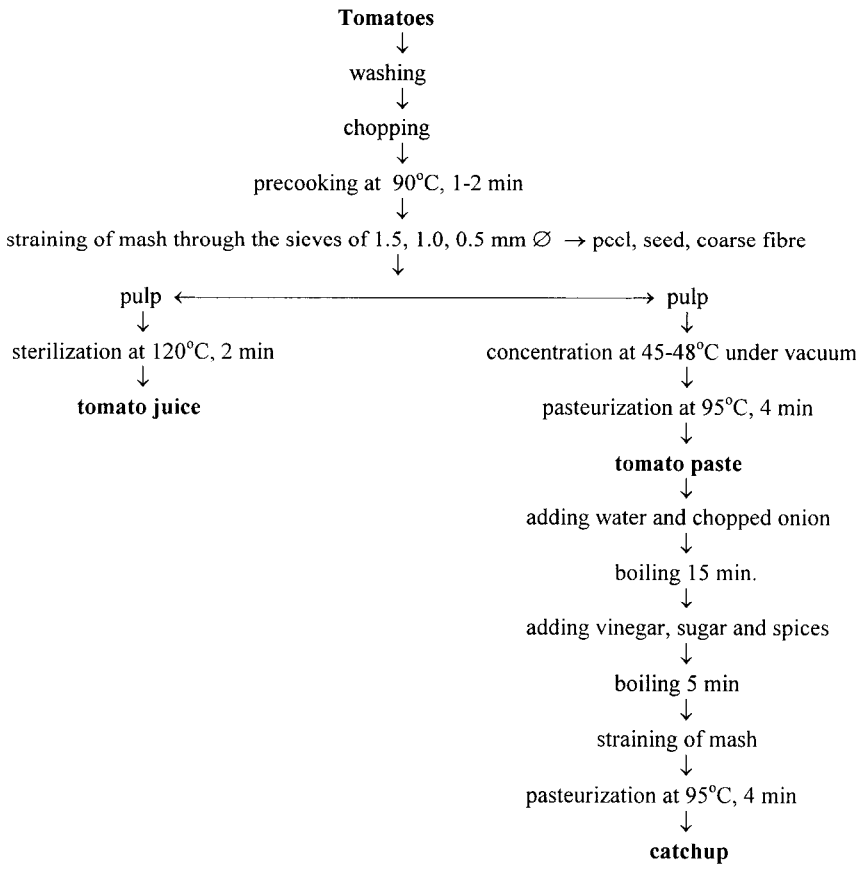

Figure 1. Schematic diagram of tomato processing

The total rain fall and mean air temperature during the growing season were recorded 70 mm and 18°C respectively. Necessary cultural works such as irrigation, fertilization, hoeing were done as in practice. Tomatoes were harvested August, September and October 1992 and stored in a deep-freeze until being analyzed.

Harvested tomatoes belonging to the same harvesting season were combined as in industrial process and sub-samples in a certain amount (~500 g) were processed into juice, paste and catchup as shown in Figure 1.

To determine the effect of processing on the residue, samples were taken after each processing step and then total chlorpyrifos residues in those samples and final products were determined by combusting of samples in a biological material oxidizer (Anonymous 1991). $^{14}CO_2$ was absorbed in an absorbent mixture and quantified by liquid scintillation counting (LSC). Food processing retention factors and concentration constants of the residue in tomato products were calculated according to Anonymous 1994 to evaluate the results in respect to acceptable daily intake (ADI). Chlorpyrifos and related residues were extracted from tomatoes, tomato juices, pastes and catchups with cold methanol and then chromatographed on silica layers by using toluene+methanol+hexane

(18:1:1 v/v) solvent system to characterize the radioactive residues. Spots were visualized by exposure to UV lamp. Rf values of chlorpyrifos and its metabolite 3, 5, 6-trichloro-2-pyridinol (TCP) were 0.88-0.92 and 0.28-0.35, respectively. For radio scanning, zones of 1 cm were scraped off the plates and suspended in *Rotiszint*. Then the activity was quantified by LSC (L'Annunziata 1979).

RESULTS AND DISCUSSION

While the theoretically applied amount of chlorpyrifos per plant was 15552 µg during growing season, the amount of chlorpyrifos residue at the last harvesting time was found 11594 µg/plant. That amount corresponds to 74.5 % of the theoretically applied dose. Table 1 shows the distribution of chlorpyrifos residue in different plant parts. Tomato fruits contain 1.1 % of total chlorpyrifos residue in the plant.

Table 1. Distribution of chlorpyrifos residue in different plant parts

Plant parts	Weight of plant part grams on the wet basis	Chlorpyrifos residue		
		µg/g	total µg	%
Leaves	4938	2.03	10024	86.4
Stem and stalks	2000	0.56	1120	9.7
Roots	600	0.54	324	2.8
Fruits	550	0.23	126	1.1
Total	8088	-	11594	100.0

Total chlorpyrifos residues in tomatoes harvested at early, mid and late seasons were determined 0.27, 0.25 and 0.18 ppm, respectively;. These residue levels in tomatoes were above the national maximum residue limits (0.2 ppm), but below the international MRL (0.5 ppm) established by Codex Alimentarius Commission (Anonymous 1989).

Table 2. Total and extractable residues in tomatoes and tomato products

Product	Total residue		Extractable residue		
	dpm/g	µg/g*	dpm/g	µg/g	%
Tomatoes, early season	668.00	0.27	550.36	0.22	82 ± 6.9
mid season	615.36	0.25	546.37	0.22	89 ± 9.9
late season	448.07	0.18	381.94	0.15	85 ± 2.1
Tomato juice, early season	656.93	0.26	617.20	0.25	94 ± 1.6
mid season	554.73	0.22	579.87	0.23	104 ± 0.1
late season	404.83	0.16	421.54	0.17	104 ± 2.1
Tomato paste, early season	3025.00	1.21	2123.00	0.85	70 ± 3.2
mid season	1732.50	0.69	1368.00	0.55	74 ± 6.8
late season	1400.00	0.56	1371.00	0.55	101± 4.9
Catchup, early season	2425.00	0.97	1766.00	0.70	73± 6.1
mid season	1125.00	0.45	822.00	0.33	73± 1.1
late season	975.00	0.39	905.00	0.36	89± 5.2

*: Residue in sample = radioactivity amount in sample (dpm/g) / specific activity of the

During the processing of harvested tomatoes to tomato juice, paste and catchup, total residue losses were in the range of 21-39 %. The most important processing step to remove residue was straining of tomato mash (Table 3). Loss rates were parallel to sediment amounts removed from tomatoes. Effects of other steps on residue losses were smaller. The reason why the residue losses were higher in catchup processing is evaporation losses due to boiling of tomato paste in open container.

Table 3. Residue losses during processing of tomatoes harvested at early, mid and late season into juice, paste and catchup

Processing steps	Residue losses, %		
	Early season	Mid season	Late season
Tomatoes	0.00	0.00	0.00
Washing	4.63	5.78	2.45
Precooking	1.43	0.49	1.21
Peel, seed,coarse fibre	12.16	19.11	17.47
Sterilization of juice	4.33	0.84	0.00
Tomato juice	**22.55***	**26.22***	**21.13***
Evaporation of pulp	2.33	1.13	0.35
Pasteurization of paste	0.00	0.89	2.23
Tomato paste	**20.55***	**27.40***	**23.71***
Tomato catchup	**35.25***	**38.79***	**31.64***

* : Total loss in final products

When the tomatoes were processed into juice, chlorpyrifos related residue concentrated 0.97 times and 3.56 times in paste. Here it can not be mentioned any reduction in residue, since tomato pulp is normally intended to concentrate 3-4 times during paste production (Table 4).

Table 4. Food processing retention factor (Fr) and processing efficiency (Pe) for tomato products

Product	Retention factor of residue [a]	Processing efficiency	Concentration constant of residue[b]	MPA[c],kg
Tomato juice, early season	0.85	0.82	1.04	-
mid season	0.78	0.85	0.92	-
late season	0.80	0.85	0.94	-
			0.97 ± 0.06	**3.13**
Tomato paste, early season	0.83	0.18	4.61	-
mid season	0.77	0.27	2.85	-
late season	0.77	0.24	3.21	-
			3.56 ± 0.93	**0.88**
Catchup, early season	0.85	1.06	0.80	-
mid season	0.89	1.37	0.65	-
late season	0.92	1.32	0.70	-
			0.72 ± 0.08	**1.22**

[a]: Retention factor=Total residue amount in product / total residue amount in raw material
[b]: Concentration constant = Fr/Pe
[c] : Maximum permissible amount of product = MPA of raw material/concentration constant

The published value for AD1 is 0.01 mg/kg body weight day. Maximum permissible intake (MPI) for chlorpyrifos is then calculated as follows:

MPI = ADI x body weight
= 0.01 mg/kg.day x 70 kg
MPI = 0.7 mg/day

In considering them, a 70 kg weight-adult can consume 3.04 kg of tomatoes (MPI/residue amount in raw material) containing 0.23 mg/kg chlorpyrifos residue. If the same person uses tomato products instead of that tomatoes, maximum permissible amount (MPA) of products are shown in Table 4. All those product amounts are above the national daily consumption level.

Extractable residues in tomatoes ranged between 82-89 %, 94.0-104.5 % in tomato juices, 70.2-101.3 % in tomato pastes and 72.8-88.6 % in catchups (Table 2). When these methanol extracts were chromatographed on thin layers of silica to characterize radioactive residues, all radioactivity remained at the spotting line. In order to verify the performance of the entire analytical procedure, spiked tomato mash and tomato products processed from that tomatoes were also extracted and then analyzed by TLC. Although some quantity of radioactivity remained at spotting line, ^{14}C-chlorpyrifos and ^{14}C-TCP could be detected (Table 5). Comparison of the data obtained from aged and spiked sample suggests that the residue at spotting line might be a conjugate of chlorpyrifos related residue

Table 5. Separation of chlorpyrifos and TCP from spiked samples

Spiked sample	Radioactive compounds, %*		
	Spotting line (Rf : 0.00)	TCP (Rf : 0.28-035)	Chlorpyrifos (Rf : 0.88-0.92)
Tomatoes	4.69	-	95.31
Tomato juice	19.76	8.98	71.26
Paste	7.82	-	92.18
Catchup	10.91	4.99	84.10

* **:** Percentage of total radioactivity applied

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