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# ANALYSIS OF IMIDACLOPRID RESIDUES IN FRUITS, VEGETABLES, CEREALS, FRUIT JUICES, AND BABY FOODS AND DAILY INTAKE ESTIMATION IN AND AROUND LUCKNOW, INDIA

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Running title: Imidacloprid residues in food commodities

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**Abstract**—A total of 250 samples—including fruits, fruit juices, and baby foods (50 samples each); vegetables (70 samples); and cereals (30 samples)—were collected from Lucknow, India, and analyzed for the presence of imidacloprid residues. The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method of extraction coupled with high-performance liquid chromatographic analysis were carried out, and imidacloprid residues were qualitatively confirmed by liquid chromatography-mass spectrometry. Imidacloprid was not detected in samples of fruit juices and baby foods. It was, however, detected in 38 samples of fruits, vegetables, and cereals, which is about 15.20% of the total samples. Of samples of fruits, 22% showed the presence of imidacloprid, and 2% of samples showed residues above the maximal residue limit. Although imidacloprid was detected in 24% of vegetable samples, only 5.71% showed the presence of imidacloprid above the maximal residue limit. However, 33% of cereal samples showed the presence of imidacloprid, and about 3% of samples were above the maximal residue limit. The calculated estimated daily intake ranged between 0.004 and 0.131 µg/kg body weight, and the hazard indices ranged from 0.007 to 0.218 for these food commodities. It is therefore indicated that lifetime consumption of vegetables, fruits, fruit juices, baby foods, wheat, rice, and pulses may not pose a health hazard for the population of Lucknow because the hazard indices for imidacloprid residues were below 1.

**Keywords**—Imidacloprid, QuEChERS method, Maximum residue limit, Estimated daily intake, Hazard index

#### **INTRODUCTION**

Neonicotinoids are an important class of pesticides now widely used in agriculture in place of persistent organochlorine pesticides due to their broad-spectrum activity, low bioaccumulation potential, and relative immobility in soil [1]. Imidacloprid is a relatively new class of neonicotinoid pesticide with a distinct mode of action [2]. Since it is a systemic chloronicotinyl insecticide that blocks the microtinergic neuronal pathway, it is used for control of sucking insects such as rice hoppers, aphids, ticks, white flies, termites, and turf insects. It is commonly used on rice, soya beans, maize, potatoes, cotton, sugar beets, and kitchen garden vegetables and fruits [3]. In the Indian market, imidacloprid is included in the trade products Gaucho, for seed treatment, and Confidor, for leaf and soil treatment. Its use as a replacement for other insecticides is increasing. It is systemic when used in seed and dressing of soil treatment [4]. Residual data on the environmental fate of imidacloprid are inconsistent because some authors consider it to be relatively immobile in soil and do not expect it to leach into groundwater [5,6]. However, some studies indicate the contrary [7]. Foliar spray and seed treatment of pesticides at different stages of cultivation and during postharvest storage play an important role in food protection and quality preservation.

Increased use of chemical pesticides has resulted in contamination of the environment and many associated long-term effects on human health, ranging from short-term impacts such as headaches and nausea to chronic impacts such as cancer, reproductive harm, and endocrine disruption [8]. Given the potential risk of pesticides for public health, their use in agriculture is subjected to constant monitoring.

Monitoring pesticide residues in food for the evaluation of food quality is a priority objective of pesticide research, to avoid possible risk to human health. Thus, periodic monitoring of pesticide

residues in food is very important to determine the judicial use of pesticides in the interest of public health. Surveillance focuses on the proper use of pesticides in terms of authorization and registration (application rates and preharvest intervals) and compliance with maximum residue limits (MRLs). Many regulatory authorities have established MRLs or tolerance levels to protect the environment and consumer health. To evaluate the safety of consumers regarding pesticide residues, the exposure needs to be assessed and compared with health safety limits or toxicological end point values such as the acceptable daily intake and the acute reference dose. The MRL is a product limit and is based on the application of pesticides on crops according to good agricultural practices in controlled field experiments. Health safety limits or toxicological end point values such milligrams per kilogram) that is legally permitted in specific food items. Exceeded MRLs are strong indicators of violations of good agricultural practices. Exposure or intake of a compound below its health safety limit is considered to be safe. The residue concentration may be above the MRL without representing a risk to the consumer [8].

Therefore, analysis of pesticide residues is important for proper assessment of human exposure. Unfortunately, negligible data are available on the contents of imidacloprid residues in food commodities sold in the local markets of the Lucknow region in India. Lucknow, the capital of Uttar Pradesh (which is the second largest state in the economy of India), is an important fruit and vegetable exporting area in northern India. The present share of Uttar Pradesh in total horticultural production of the country is approximately 26%. Uttar Pradesh ranks third in fruit, second in vegetable, and first in potato production in India. Therefore, assessing the risk of pesticide residues in these commodities intended for human consumption is necessary. The Lucknow region is one of the largest agricultural areas located in northern India and known as a major contributor to the

national food grain stock. Wheat, rice, pulses, oil seeds, and potatoes are the major agricultural products.

In view of the large-scale use of imidacloprid and the scarcity of Indian literature [3,9], it is essential to assess the present environmental load of imidacloprid residues in different food commodities because imidacloprid is a toxic chemical [10–14]. We therefore analyzed imidacloprid residues in vegetable, fruit, cereal, fruit juice, and baby food samples in and around Lucknow during 2010 and 2011. We also determined the estimated daily intake (EDI) of imidacloprid in the local population of Lucknow from consuming the above food commodities.

## **MATERIALS AND METHODS**

#### Chemicals

Acetonitrile, acetone, and n-hexane (high-performance liquid chromatography [HPLC] grade) were purchased from Sigma-Aldrich. The solvents were glass-distilled before use. Anhydrous magnesium sulfate (MgSO<sub>4</sub>; Himedia) was purified with acetone and baked for 4 h at 600°C in a Muffle Furness to remove possible phthalate impurities. The primary secondary amine bondasil, 40 µm parts 12213024 of Varian, was used for sample preparation. C-18 cartridges were procured from United Chemical Technology. Imidacloprid standard was procured from Supelco Sigma-Aldrich.

#### Sample collection

A total of 250 samples of five food commodities (vegetables, fruits, cereals, fruit juices, and baby food), comprising 10 samples of each, were collected from October 2010 to July 2011 from the local markets of Lucknow. The selected fruits were apple, banana, orange, grapes, and pomegranate; vegetables were cabbage, cauliflower, tomato, potato, okra, brinjal, and capsicum; and cereals were wheat, rice, and pulses. Fruit juices were mango, guava, pineapple, orange, and lychee; and baby

foods were coded as BF-1 to BF-5. The ingredients of the baby foods (BF1–5) are shown in the footnote of Table 1. Samples were collected in polythene bags, transported to the laboratory, and analyzed as soon as possible or stored at 4°C until analysis.

#### Extraction and cleanup

Samples (100 g of vegetables, fruits, cereals) were chopped and ground in a Waring blender separately. Macerated samples (10 mg) or fruit juices (10 ml) were taken for imidacloprid residue analysis using the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method with slight modifications [15,16]. Macerated samples (10 g) of each commodity were mixed with 10 ml acetonitrile and 4 g of anhydrous MgSO<sub>4</sub> in a centrifuge tube and shaken for 10 min at 50 rpm in a rotospin test tube mixture. The extract was centrifuged for 10 min at 10,000 rpm. Supernatant was collected and evaporated to dryness under a slow stream of nitrogen at 40°C. Dried extracts were reconstituted with 1 ml of acetonitrile. A further 1 ml of extract was cleaned with the mixture of 50 mg primary secondary amine, 150 mg anhydrous MgSO<sub>4</sub>, and 10 mg activated charcoal. The extract was again shaken for 10 min at 50 rpm on a rotospin (Tarson test tube mixture) and centrifuged for 10 min at 10,000 rpm. Clean supernatant was collected for HPLC analysis.

Samples of baby food were extracted with the same procedure as above, but extracts were cleaned by the solid-phase extraction method. The concentrated extract was then loaded onto  $C_{18}$  solid-phase extraction cartridges prewashed with n-hexane for the cleanup process. Cartridges were then eluted with acetonitrile. Elutes obtained after column cleanup were filtered through a 0.22-µm Millipore filter and subjected to analysis. Cleaned extract (50 µl) was injected into HPLC with a photodiode array for residue analysis [17].

#### HPLC analysis

The aliquot of final sample extracts was analyzed on an HPLC system (515 series; Waters) equipped with a photo diode array detector (model 996; Waters) using a reversed-phased, C-18 ODS analytical column (75  $\cdot$  4.6 mm inner diameter, 3.5  $\mu$  particle size), with a precolumn of the same phase (both supplied by Waters). The HPLC system consisted of a binary pump, an online degasser, thermostatic column housing, and Empower<sup>2</sup> chromatography manager software. The solvent system that constituted the mobile phase was acetonitrile and water (20:80, v/v). The flow rate was maintained at 1.0 ml/min in isocratic mode throughout the analysis, and the injection volume was 50  $\mu$ l. Chromatograms were extracted at 270 nm using the photodiode array. Residues were further confirmed by liquid chromatography–mass spectography (LC-MS)

## LC-MS analysis

We carried out MS using a Waters ZQ 2000 single quadrupole mass spectrometer with an electrospray ionization performed in positive and negative mode. Full-scan spectra were recorded from m/z 100 to 500 at a scan time of 0.5 s and an interscan delay of 0.1 s. Mass spectra were represented by centroid mode. The main other instrumental settings were capillary voltage 3.5 kV, cone voltage 30 V, extractor 5 V, ion energy 0.1, source temperature 150°C, desolvation temperature 300°C, cone gas (N<sub>2</sub>) flow rate 0 L/h, and desolvation gas (N<sub>2</sub>) flow rate 300 L/h. Selected-ion monitoring of the most abundant ion was used for quantification. The MS detector was tuned for the maximum sensitivity of the parent ion at m/z 256 and of the product ions at m/z 209 and 175.

# Recovery studies and quality control

Imidacloprid was identified by matching the retention time of the sample with its external standard. Procedural blanks consisting of all reagents and glasswares used during the analysis were

periodically determined to check for cross-contamination. Since no compounds that interfere with the sample were detected, values were not corrected for procedural blanks. Absolute recovery of imidacloprid was measured by analyzing three samples of each commodity fortified at 0.0625, 0.125, 0.25, and 0.50 mg/kg, which indicated that overall recovery ranged from 77.5 to 111% and the relative standard deviation from 5.22 to 14.20% for all commodities.

An imidacloprid calibration curve was generated, and the linear relationship was evaluated across the range of expected sample concentrations. Linearity was obtained by a linear regression plot of known concentration versus response using a minimum of four different concentrations of imidacloprid. Residues were evaluated across at least three separate runs from different concentrations of imidacloprid. The regression equation was y = 16,627x + 158.70, with  $r^2 = 0.999$ . The limit of detection and limit of quantification for imidacloprid in the present study were obtained in the range of 0.004 to 0.01 and 0.014 to 0.07 mg/kg, respectively.

#### **RESULTS AND DISCUSSION**

#### Analysis of imidacloprid in different food commodities

A total of 250 samples were analyzed. Samples of fruits, fruit juices, and baby foods (50 samples each); vegetables (70 samples); and cereals (30 samples) were analyzed for the presence of imidacloprid residues. Imidacloprid was not detected in samples of fruit juices and baby foods. Similarly, apple and peach juice of Turkey and sugarcane juice of Brazil have shown no pesticide residues [18,19]. However, some studies have reported traces of pesticides in fruit-based soft drinks of Spain [20]. Thus, these two food commodities may be free from imidacloprid due to their processing. However, imidacloprid was detected in 38 samples of fruits, vegetables, and cereals, which represents around 15.20% of the total samples. Imidacloprid was observed in 22% of fruit samples, and 2% of samples were above the MRL.

The level of imidacloprid ranged from 0.02 to 0.26 mg/kg in apples, from levels not detectable to 0.04 mg/kg in bananas, from 0.019 to 0.13 mg/kg in oranges, and from 0.04 to 0.78 mg/kg in grapes (Table 1). Imidacloprid was not detected in any of the samples of pomegranates. However, one sample of grapes showed imidacloprid residue above the MRL (0.50 mg/kg).

Although imidacloprid was detected in 24% of vegetable samples, only 5.71% showed imidacloprid above the MRL

(http://www.codexalimentarius.net/pestres/data/index.html;jsessionid=8D32B185DD5A990D78989<u>CA0D54379E5</u>). The presence of imidacloprid residues was in the following order: potato > cauliflower > cabbage > tomato > brinjal > capsicum > okra. Of cereals, 33% of samples showed imidacloprid, but only 3% of samples were above the MRL (0.05 mg/kg).

Imidacloprid residues were also reported in vegetables and fruits of Palestine. The imidacloprid concentration in several crops of Palestine was found to exceed the MRL [21]. Fernandez-Alba et al. [22] found imidacloprid residues in 25 to 53% (average 21%) of 200 samples of fruits and vegetables analyzed from Almeria, Spain.

Many countries have legal directives to control levels of pesticides in food through the MRL, to protect consumers' health (http://www.who.int/foodsafety/publications/chem/regional\_diets) [23,24]. The level of pesticide residues in food commodities are legislated to minimize exposure of consumers to unnecessary intake of pesticides and to ensure their judicious use. From a potential health perspective, it is necessary to compare exposure estimates to find out toxicological criteria such as EDI.

The results of the present study have been used to calculate the EDI, expressed as microgram pesticides per kilogram body weight. The EDI is a real estimate of pesticide exposure that was calculated as per the international guidelines [25,26] using the following equation:  $EDI = \sum C \times F/D$ 

 $\times$  W, where C is the average imidacloprid concentration in each commodity (micrograms per kilogram), F is the mean annual intake of food per person (kilograms), D is the number of days in a year (365), and W is the mean body weight (60 kg).

The annual intake per person of fruits, vegetables, wheat, rice, and pulses were 9.5, 23, 52.8, 66, and 12 kg, respectively, according to an Indian survey performed in the years 1975 to 2000 and 2005 to 2006 (http://mospi.nic.in/mospi\_nsso\_rept\_pubn.htm) [27,28]. The average level of imidacloprid in each food commodity, the annual consumption of individual commodities per person, the EDI, and acceptable daily intakes (micrograms per kilogram body weight) established by the Food and Agriculture Organization and the World Health Organization [29,30] are compared in Table 2. The EDIs have been calculated between 0.004 and 0.131  $\mu$ g/kg body weight, while the hazard indices (EDI/acceptable daily intake) ranged from 0.007 to 0.218 for the tested commodities.

It is therefore indicated that lifetime consumption of vegetables, fruits, fruit juices, baby foods, wheat, rice, and pulses may not pose health hazards for the population of Lucknow because the hazard indices for imidacloprid residues were below 1 [31]. It is reported that common home processing removes significant amounts of pesticides from vegetables [32]. Therefore, the health hazard due to imidacloprid residue in vegetables, fruits, fruit juices, baby foods, wheat, rice and pulses may not be of great concern. Monitoring of pesticide residues in food is a priority objective for ensuring compliance with good agriculture practices and their judicial use and to avoid possible risk to human health.

#### CONCLUSION

The present study showed a mild occurrence of imidacloprid residues in the analyzed food commodities collected from Lucknow. It is therefore indicated that long-term consumption of vegetables, fruits, fruit juices, baby foods, wheat, rice, and pulses may not pose a health hazard for the population of Lucknow because the hazard indices for imidacloprid residues were below 1. The findings also suggest that periodic monitoring of imidacloprid residues should be carried out in other food commodities at the national level in view of the possible human health risk.

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Table 1. Mean levels, concentration range, and frequencies of imidacloprid residues in different food commodities <sup>a</sup>

Commodity	Sample	Mean	Residue range	No. samples		No. samples
		(mg /kg)	(mg /kg)			above MRL
				Analyzed	Detected	(mg/kg)
Fruits	Apple	$0.04\pm0.02$	0.02–0.26	10	3	0
	Banana	$0.005\pm0.004$	ND-0.04	10	1	0
	Orange	$0.02\pm0.01$	0.019–0.13	10	4	0
	Grapes	$0.13\pm0.08$	0.04–0.78	10	3	1 (0.5)
	Pomegranate	ND	ND	10	0	0
Vegetables	Cabbage	$0.15\pm0.11$	0.08–0.89	10	3	1 (0.5)
	Cauliflower	$0.21\pm0.12$	0.29–0.93	10	3	1 (0.5)
	Tomato	$0.08\pm0.06$	0.24–0.46	10	2	0
	Potato	$0.32\pm0.17$	0.19–1.32	10	4	2 (0.5)
	Okra	$0.01\pm0.01$	ND-0.11	10	1	0
	Brinjal	$0.06\pm0.03$	0.13-0.21	10	3	0
	Capsicum	$0.05\pm0.05$	ND-0.45	10	1	0
Cereals	Wheat	$0.01\pm0.01$	0.01–0.10	10	3	1 (0.05)
	Rice	$0.009\pm0.005$	0.01-0.05	10	4	0
	Pulses	$0.008\pm0.005$	0.016-0.05	10	3	0
Fruit juices	Mango	ND	ND	10	0	0
	Guava	ND	ND	10	0	0
	Pineapple	ND	ND	10	0	0

+						17	
	Orange	ND	ND	10	0	0	
	Lychee	ND	ND	10	0	0	
Baby foods <sup>b</sup>	BF-1	ND	ND	10	0	0	
	BF-2	ND	ND	10	0	0	
	BF-3	ND	ND	10	0	0	
	BF-4	ND	ND	10	0	0	
	BF-5	ND	ND	10	0	0	
Gross total				250	38	6	

<sup>a</sup> Values represent mean ± standard error of 10 samples. Values in parentheses indicate MRL (CODEX, 2005).

<sup>b</sup> Ingredients of baby foods (BF): BF-1, wheat and milk; BF-2, apple, wheat, and milk; BF-3, banana, wheat, and milk; BF-4, honey, wheat, and milk; BF-5, rice, wheat, and milk.

MRL = maximum residue limit; ND = not detected.

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 Table 2. Calculation of estimated daily intake and hazard index of imidacloprid in different food

 commodities

Commodity	Average	Mean	No.	Average	ADI	EDI	Hazard
	imidacloprid	annual	days in	weight of	(µg/kg bw	(µg/kg	index
	concentration	intake of	year	person	daily)	bw	(%)
	in commodity	commodity	(D)	(W)		daily)	
	$(\sum C) (\mu g/kg)$	per person					
		(kg)(F)					
Fruits <sup>a</sup>	39	9.5	365	60	60	0.016	0.028
Vegetables <sup>a</sup>	125	23	365	60	60	0.131	0.218
Wheat <sup>b</sup>	10	52.8	365	60	60	0.024	0.040
Rice <sup>b</sup>	9	66	365	60	60	0.027	0.045
Pulses <sup>b</sup>	8	12	365	60	60	0.004	0.007

<sup>a</sup>From National Nutrition Monitoring Board [27].

<sup>b</sup>From National Sample Survey Organization [28].