

Health Status Among Pesticide Applicators at a Mango Plantation in India

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Abstract

Observations of mango plantation workers applying chemicals showed many were mixing pesticides without the appropriate personal protective equipment. Personal hygiene was lacking in that many applicators commonly ate and drank without previously washing their hands. Medical evaluation of thirty-four of these workers at a free health clinic shows pesticide exposure may be linked to health problems. Respiratory, gastrointestinal, ocular and dermal problems were observed; biochemical analysis shows decreased glutathione levels and increased levels of malondialdehyde thereby suggesting significant pesticide exposure. Our study clearly indicates that growers and workers applying pesticides in mango plantations need additional training on how to properly and safely use pesticides.

Keywords: health, occupational, safety, PPE, exposure, pesticide, medical, monitoring

Introduction

Pesticides have been heavily promoted as a means to increase agricultural productivity and eradicate many vector-borne diseases. India is the world's largest mango producer, growing nearly 1000 varieties in an area of 1.23 million hectares. India's annual production of 10.99 million tons accounts for 57.18% of the total world mango production. The

cultivated mango is a natural hybrid between *M. indica* and *M. sylvatica* occurring from southeastern Asia to India. The varieties of mango grown in Uttar Pradesh (where the present study was undertaken) include Dashehari and Chausa (Anonymous, 2005).

Pests of mango include mealy bug, mango hoppers and mango scale. Mealy bug does a lot of damage during the flowering and fruiting

stages, January through April, when young nymphs crawl up the trees and congregate on growing shoots and panicles. Methyl parathion 50 gm dust (Follidol 2%) is used to kill the nymphs. Mango hoppers are active during February and March, at the time of flowering. The nymphs and adults suck the sap from tender leaves and panicles, which become sticky and sooty. Two sprays of insecticides are essential to reduce the menace; the first, at the end of February, and the second, at the end of March, using 500 gm of carbaryl (Hexavin 50WP) or 400 ml of malathion 50EC or 350 ml of endosulfan (Thiodan 35EC), in 250 litres of water. Mango scale inflicts damage by sucking the sap from leaves. Spraying 300 ml of methyl parathion 50EC in 500 litres of water in March can reduce the infestation (Noatay, 2003).

Pesticides are dispersed in water and then sprayed manually on mango trees using mechanized sprayers.

Figure 1. Spraying Equipment.



Figure 2. Spraying Equipment.



Spraying starts at the end of December and continues until March, but if the pests remain uncontrolled, spraying is continued. Spraying is generally done by diluting 200 ml of liquid pesticide with 200 litres of well water. At 6.30 a.m., workers load the pesticide into the spray tank and at 7 a.m. they start spraying plantations using a spraying tube. Pesticide application periods last for 3 to 4 hours per week and the spraying operation covers an average of 0.5-hectare area per day.

See Figures 1 to 4 for photographs of equipment, mixing, loading, and application.

Figure 3. Mixing and Loading.



Figure 4. Application.

A health study conducted by the Industrial Toxicology Research Centre (ITRC) in Lucknow during 1990, on pesticide applicators working in Malihabad mango plantations, showed overall morbidity rates of 42.8%. Through their work subjects were exposed to mixtures of monocrotophos, phosphamidon, dichlorvos, oxydemeton methyl, malathion, endosulfan, methyl parathion, dimethoate or carbaryl. The chief morbidities were respiratory disorders (33.4%) and musculoskeletal disorders (15%) and those disorders pertaining to the central nervous system (6%), predominantly polyneuropathy (Anonymous, 1990). Exposure to organophosphates and N-methyl carbamate insecticides are known to lead to acute systemic effects due to cholinesterase inhibition, which ultimately leads to overstimulation and depression of the nervous system (Yuknavage et.al, 1997). Early symptoms of acute poisoning include weakness, nausea, vomiting, excessive sweating, salivation,

headache, skin rashes, ocular problems and difficulty in walking (Mehler et al, 1992). Later symptoms of severe poisoning may include unconsciousness, pulmonary edema, respiratory failure and death. Even a single episode of organophosphate intoxication has been associated with a persistent decline in neuropsychological functioning (Rosenstock et al, 1991). However, firm conclusions on neuropsychological effects of chronic exposure to pesticides are difficult to draw since information is scarce, particularly in developing countries (London et al, 1998). Both acute and chronic effects are of great concern. However, chronic effects, including neurological and reproductive effects and cancer are more difficult to ascertain, although some studies have found associations between pesticide exposure and these chronic effects (Blair et al, 1993).

Mango orchards around Lucknow, India, were selected as a study site for several reasons. A previous study (Anonymous, 1990) provided baseline information. Mangos have traditionally been a major cash crop in the area. Applicators and their assistants are actively engaged in mixing and spraying, and live in an area where trees are systematically treated throughout the year, and a large number and wide variety of pesticides are used in mango production.

New pesticides have been introduced into the market since the 1990 study (e.g. cypermethrin formulations, quinalphos and deltamethrin). The aim of this study is to assess the present health status of pesticide applicators after the

introduction of new pesticides and new spraying techniques adopted since 1990. This study will also focus on biological monitoring studies in pesticide applicators, i.e., estimation of glutathione, estimation of lipid peroxidation and acetylcholinesterase assay estimation.

Procedures

A health examination was offered free of charge to pesticide applicators working in mango plantations in Uttar Pradesh, India. Each pesticide applicator was asked to sign a consent form verifying their willingness to participate in the study. In return, each volunteer received a report of the physical examination results. ITRC personnel organized a health screening camp. A total of thirty-four pesticide applicators, ranging between 20- 25 yrs old, volunteered to be medically evaluated. All of the applicators that volunteered were male. The health camp (Figure 5) was conducted in 2004, during the months of January and February, while the pesticide application season was underway.

Figure 5. Meeting with Applicators.



Medical officials initially conducted and recorded visual observations of each of the pesticide applicators while they performed their daily activities to assess whether pesticides contacted an applicator's clothing or body. They also recorded their method of handling pesticides, the condition of the application equipment, and eating, drinking, and personal cleanliness habits. Each of the applicators was provided with a report that summarized the findings of the observers along with precautionary measures that were tailored to each applicator.

Each of the pesticide applicators who volunteered was given a complete clinical examination including general observations and a physical examination of the central nervous, respiratory, cardiovascular, gastrointestinal, ocular, skin and musculoskeletal systems. Physical examination of pesticide applicators was conducted in accordance with recommendations outlined in the Declaration of Helsinki (Anonymous, 1983). The average time taken to provide the full medical check up was approximately 10 min. Any health problems observed during the physical and general examination of applicators were recorded in the survey questionnaire by the investigator. The control population for the biochemical comparative study was selected from age- and socio economic status-matched volunteers from the same locality not occupationally exposed to pesticides.

Lung function test

Peak Expiratory Flow Rate (PERF) of each applicator was performed

using a Peak flow meter (Instrument Model: Clement Clarke Inc, USA). The purpose of the lung function test (Figure 6) was to evaluate bronchial obstruction.

Figure 6. Lung function test.



PEFR tests were performed during the morning hours between 6:00 and 8:30 a.m. The lung function test was performed on each volunteer without a nose clip. The volunteer performed the lung function test three times allowing for sufficient rest between repetitions. The bronchial condition of each volunteer was classified as a mild, moderate and severe obstruction (Rastogi et al., 1989).

Estimation of glutathione

Glutathione was used as a surrogate measurement for oxidative stress due to pesticide toxicity. Glutathione in the blood was measured by obtaining a 0.5 ml blood sample from each volunteer. The blood was mixed with 1.5 ml of water, which was then added to 2 ml of 10% trichloroacetic acid (TCA). This mixture was centrifuged at 2000 rpm for 15 min. One ml of supernatant was added to 4 ml of 0.1 M phosphate buffer (pH 7.4) and 0.1 ml of 0.4% of DTNB (5,5-dithiobis (2-nitrobenzoic acid) in a phosphate buffer. The optical density was read

at 412 nanometers according to the procedures outlined by Jallow et al. (1974).

Estimation of lipid peroxidation

Lipid peroxidation was measured using malondialdehyde (MDA) in blood to determine the oxidative stress in pesticide applicators. Phosphate buffer (pH 7.4; 0.1 M) was added to 0.5 ml of blood and incubated for 30 minutes at 37°C. This was centrifuged and 3 ml of supernatant was collected. One ml of 1% tribromoacetic acid (TBA) was added to the supernatant then the mixture was placed in a boiling water bath for 15 minutes. Contents were cooled in ice water and centrifuged for 15 minutes at 2500 rpm. Optical density was taken against a suitable blank at 532 nanometers and was converted to the equivalent of MDA (nmol/ml blood) using a molar extinction coefficient of $1.56 \times 10^5 \text{ mol/L}^{-1} \text{ cm}^{-1}$ (Stocks and Dormandy, 1971).

Acetylcholinesterase assay estimation

Acetylcholinesterase activity in blood was determined for each individual as an indicator of cholinesterase activity in the blood and was estimated using the method of Ellman et al. (1961). The four ml total volume of incubating mixture consisted of 0.1 M Tris HCl Buffer (pH 7.4), 1.0 mM acetylthiocholine iodide, 0.025 ml of 25X diluted blood and 0.015 ml of incubating mixture buffer - Tris HCl 0.1 M, (pH 7.4). The assay was incubated for 15 minutes with shaking at 37°C. The reaction was then stopped with a mixture of DTNB (5, 5-dithiobis (2-nitrobenzoic acid) and SDS (sodium

dodecylsulfate). The absorbance was read at 412 nanometers and was converted to an equivalent of m moles hydrolyzed using a molar extinction coefficient of $13600 \text{ mol/L}^{-1} \text{ cm}^{-1}$.

Statistical analysis

Age adjusted odds ratio of the prevalence of bronchial obstruction in the ≥ 5 yrs exposure group when compared to the ≤ 5 yrs exposure group was calculated. Its significance was tested using multiple logistic regression analysis where the independent variables were exposure years (binary, ≤ 5 yrs and ≥ 5 yrs) and age (continuous) and the outcome variable was dependent. Significance of mean values of the biochemical parameters in the exposed and

control workers were compared using a student 't' test where the variance between the groups were homogeneous. For heterogeneous variances, the Beharan and Fischer modified 't' test was used.

Results

Reported observations of applicator behavior during pesticide applications were: mixing chemicals with bare hands, pesticide leakage from tanks during spray operations, wetting of bare skin and clothes with hazardous pesticides, and taking food without previously and properly washing with water after finishing an application.

The morbidity profiles for the pesticide applicators are given in Table 1.

Table 1. Morbidity Pattern of Pesticide Applicators.

System	n	(n = 34)
		%
Respiratory	11	32.4
Ocular	3	8.8
Gastrointestinal	6	17.6
Dermal	8	23.5

Prevalence of respiratory problems was reported in 32.4% of the volunteers, the most common being chest discomfort and tightness, productive and dry cough, dyspnea and basal crepitation of both lungs. Subjects did not report any symptoms associated with the central nervous and musculoskeletal system. Prevalence of gastrointestinal problems were reported in 17.6% of the applicators and included stomach cramps and epigastric abdominal pain. (The epigastrium is the upper part of the abdomen that lies within the angle of

the ribs over the stomach.) Prevalence of dermal problems was reported in 23.5% of the cases, primarily burning, itching and rashes on the face. Prevalence of ocular problems was reported among 8.8% of the applicators; the only ocular symptom reported was burning and stinging sensations in the eyes.

Morbidity rates in relation to length of exposure and significance of age adjusted odds ratio of the over 5 years exposure group compared to the less than 5 years exposure group are presented in Table 2.

Table 2. Morbidity Pattern of Pesticide Applicators Based on Years of Exposure.

System	≤ 5yrs group		≥ 5 yrs group		Crude Odds Ratio	Age Adjusted Odds ratio	95% CL LCL-UCL	P Value
	(n=22)		(n=12)					
	n	%	n	%				
Respiratory	4	18.2	7	58.3	6.3	7.9	1.3-47.3	0.02
Ocular	1	4.8	2	15.4	4.2	37.8	0.7-2035.8	0.07
Gastrointestinal	2	9.5	4	30.8	5.0	6.9	0.8-56.1	0.07
Dermal	6	27.2	2	16.7	0.7	0.5	0.1-3.1	0.4

The prevalence of respiratory morbidity was significantly higher (age adjusted odds ratio = 7.9) in the higher exposure group compared to the less than 5 years exposure group. A non-significant trend for more gastrointestinal and ocular problems was found in the higher exposure group (≥ 5 yrs) compared to

the lower exposure (≤ 5 yrs) group. No association of skin problems with length of exposure was established.

While bronchial obstructions were observed, the length of time that a person was employed as a pesticide applicator was not significant.

Table 3. Period of Exposure and Prevalence of Bronchial Obstruction in Pesticide Applicators.

Type	≤ 5yrs group		≥ 5 yrs group		Crude Odds Ratio	Age Adjusted Odds ratio	95% CL LCL-UCL	P Value
	(n=22)		(n=12)					
	n	%	n	%				
Mild	3	14.1	3	23.1	2.1	3.2	0.4-246	0.2
Moderate	2	9.5	1	7.7	0.9	1.9	0.1-32.4	0.6
Severe	2	9.5	1	7.7	0.9	0.8	0.1-11	0.9
Overall	7	33.3	5	38.5	1.5	1.5	0.3-7.7	0.5

Table 4. Biochemical Profiles of Pesticide Applicator and Control Populations.

Parameters	Control (n=17) Mean ± SD (Range)	Applicators (n=34) Mean ± SD (Range)	P Value
Acetyl cholinesterase activity (mmol/h/L blood)	696.7 ± 100.8 (518.1-862.6)	567.9 ± 103.3 (383.5-801.9)	<0.001
Glutathione level (µg/ml blood)	280.6 ± 133.1 (114-560)	252.3 ± 104.0 (114-527)	NS*
Malondialdehyde (nmolTBARS/ml blood)	10.56 ± 2.1 (7.43-14.82)	43.21 ± 15.1 (20.62-78.15)	<0.001

*NS - non significant

AchE (acetyl cholinesterase) levels were found to be significantly decreased in the pesticide applicators tested. In addition, malondialdehyde levels were

significantly elevated among the pesticide applicators. No changes were observed in glutathione levels.

Conclusions

The use of pesticides in mango production is an important input by farmers. However, pesticide use may also present health concerns to those making the applications. Results of this study show that pesticide applicators did complain frequently of gastrointestinal, dermal and respiratory problems. Comparisons of respective symptom rates reported in 1990 vs. this study are as follows: gastrointestinal (34.7% vs. 17.6%), respiratory (33.4% vs. 32.4%), musculoskeletal (15% vs. 0%), central nervous system (6% vs. 0%), dermal (3.6% vs. 23.5%), and ocular (4.9% vs. 8.8%). Compared to 1990, pesticide applicators at mango plantations still have health risks of gastrointestinal, respiratory, dermal, and ocular morbidity due to pesticide exposure.

Measurements of biochemical parameters in the blood of applicators also suggest that their exposure to pesticides may contribute to decreases in acetylcholinesterase activity and increases in malondialdehyde, both surrogate measurements for adverse effects from pesticide exposures. The small number of pesticide applicators tested was one of the limitations of this study.

Observations of how pesticides were handled provided very insightful, instructional and meaningful inputs. Applicators were observed mixing pesticides without appropriate safety equipment. Some pesticide application equipment was in poor condition and leaking. Applicators were observed eating and drinking without first having washed their hands.

Obviously, pesticide applicators need better training in handling pesticides. We recommend the following actions

that plantation owners should immediately undertake to strengthen the safe use of pesticides.

- (a) Choose formulations of products that minimize exposures (e.g. granules).
- (b) Use closed transfer systems for loading spray equipment.
- (c) Use wide necked containers for pesticide concentrates to reduce spillage
- (d) Enclose cabs on tractors.
- (e) Provide personal protective equipment including gloves, boots, coveralls, face shields and respirators, and training in their use.
- (f) Train workers how to handle pesticide-contaminated clothing.
- (g) Use agricultural pesticides under the supervision of persons with appropriate formal training or experience.
- (h) Keep children and other bystanders away from spraying operations to protect them from exposure.

Pesticide applicators and public health authorities must become aware of the importance of protective equipment, periodic health examinations and reduced environmental pollution in order to lessen occupational risks to applicators and promote improved life conditions. Because of their intrinsically hazardous nature, pesticides must be carefully applied. This in itself can make a valuable contribution toward increasing agriculture production with no health risk to the applicator.

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References

Anonymous. 1983. Declaration of Helsinki. Ethical principles for medical research involving human subjects. World Medical Association. [<http://www.wma.net/e/policy/b3.htm>]

Anonymous. 2005. India Agro Industry, Plant Products-Mango. [<http://www.agriculture-industry-indica.com/agricultural-commodities/mango.html>]

Anonymous. 1990. Report on epidemiological health survey on pesticide sprayers. Industrial Toxicology Research Centre, Lucknow, India.

Blair, A., Dosemeci, M., & Heinmen, E.F. 1993. Cancer and other causes of death among male and female farmers from twenty three states. *Am J Ind Med.* 23: 729-742.

Ellman, G. L., Curtney, K. D., Andrews, V., & Featherstone R. M. 1961. A new and rapid colorimetric determination of acetyl cholinesterase activity. *Biochem Pharm;* 7: 88-95.

Jallow, D. J., Mitchell, J. R., Zampaglione, N., Gillote, J. R. 1974. Bromobenzene induced liver necrosis: Protective role of glutathione and evidence for 3,4-bromobenzene oxide

as the hepatotoxic metabolite. *Pharmacology* 11: 151-169.

London, L., Nell, V., Thompson, M., & Myers, J. E. 1998. Effects of long-term organophosphate exposures on neurological symptoms, vibration sense and tremor among South African farm workers. *Scand J Work Environ Health* 24: 18-29.

Mehler, L., O'Malley, M. A., & Krieger, R. I. 1992. Acute pesticide morbidity and mortality. *California Rev Environ Contam Toxicol* 129:51-66.

Noatay, K. L. 2003. Tree talk. The bat wood from cold climes - Mango. The tribune-online edition, Chandigarh [<http://www.tribuneindia.com/2003/20030210/agro.htm>].

Rastogi, S. K., Gupta, B. N., Husain, T., Mathur, N., & Garg, N. 1989. Study on respiratory impairment among pesticide sprayers in mango plantations. *Am J Ind Med.* 16: 529-538.

Rosenstock, L., Keifer, M., Daniell, W. E., McConnell, R., & Claypoole, K. 1991. Chronic central nervous system effects of acute organophosphate pesticide intoxication. *Lancet* 338: 223-227.

Stocks, J. & Dormandy, T. L. 1971. Auto-oxidation of red cell lipids induced by hydrogen peroxide. *Br. J. Haematol.* 20: 95-111.

Yuknavage, K. L., Fenske, R. A., Kalman, D. A., Keifer, M., & Furlong, C.E. 1997. Simulated dermal contamination with capillary samples and field cholinesterase biomonitoring. *J Toxicol Environ Health* 51:35-55.