Survey performa
Op pesticide poisoning
Structured questionare

1. Name of Village:
2. Name age sex weight

3. Profession:
   a. Agricultural:
   b. Non-agricultural:

4. Period of exposure:
5. Socio-Economic status:
6. Family details (no of children) Male Female

7. Type of OP pesticides use: OP OC
8. Op pesticides:
   a. Name:
   b. Frequency of spray:
   c. Single use:
   d. Combination of op pesticides use:
9. Route of exposure:
   Occupational  Para-occupational
   Daily  yes/no
   Intermittent  yes/no
   Dermal
   Ingestion
   Inhalation

9. Storage of pesticides in home:

10. distance of home from the site of pesticide (O.P):

11. Crop sprayed:

12. Specific symptoms reported:
   a) Neurological symptoms:
      Headache  yes/no
      Irritability  yes/no
      Confusion  yes/no
      Insomnia  yes/no
      Dizziness  yes/no
      Anxiety  yes/no
      Weakness  yes/no
      Nausea  yes/no
      Heart burn  yes/no
      Tiredness  yes/no

13. Gastrointestinal symptoms
      Vomiting  yes/no
      Abdominal pain  yes/no
      Diarrhea  yes/no
      Absence of appetite  yes/no
      Poor digestion  yes/no
14. Any other symptoms

Excessive lacrimation

Excessive salivation

15. Any past illness

16. Protective devices used

A. Hand gloves

B. face mask

C. any other

17. Precautions taken after using pesticides

a. Hand washing

b. cloth washing

c. other

18. Any other information

a. skin allergic manifestations:


19. Biochemical investigations

a. Hb value:

b. TLC and DLC

c. Total count of RBC

d. Total count of WBC.

e. AchE levels (blood):

f. BchE levels (blood):

g. Glutathione (blood):

h. MDA levels (blood)

i. Urinary alky phosphates:

j. Lung respiration values:

k. Nerve conduction values:
DETAILED INTERVIEW FOR OCCUPATIONAL AND ENVIRONMENTAL EXPOSURES

1. ADULT PATIENT:

Occupational exposure

- What is your occupation
- How long have you been doing this job?
- Describe your work and what hazards you are exposed to (pesticides, solvents or other chemicals, dust, fumes, metals, fibers, radiation, biologic agents noise, heat, cold, vibration).
- Under what circumstances do you use protective equipment? (work clothes, safety glasses, respirator, gloves, and hearing protection)
- Do you smoke or eat at the work site?
- List previous jobs in chronological order, include full and part-time, temporary second jobs, summer jobs, and military experience.

ENVIRONMENTAL EXPOSURE HISTORY:

- Are pesticide (e.g. bug or weed killers, flea and tick sprayers, collars, powders or shampoos) used in your home or garden or on your pet?
- If pesticides are used
- Is a licensed pesticide applicator involved?
- Are children allowed to play in areas recently treated with pesticides?
- Where are the pesticides stored?
- Is food handled properly (e.g. washing of raw fruits and vegetables)?
- Did you ever live near a facility which could have contaminated the surrounding area (e.g. mine plant smelter, dump site)?
- Have you ever changed your residence because of a health problem?
- Does your drinking water come from a private well, city water supply, and or grocery store?

**SYMPTOMS AND MEDICAL CONDITIONS**

- Does the timing of your symptoms have any relationship to your work hours?
- Has anyone else at work suffered the same or similar problems?
- Does the timing of your symptoms have any relationship to environmental activities listed above?
- Has any other household member or nearby neighbor suffered similar health problems?

**NON OCCUPATIONAL EXPOSURES POTENTIALLY RELATED TO ILLNESS OR INJURY**

- **Do you use tobacco?** If yes what forms (Cigarettes, pipe, chewing, tobacco)? About how many do you smoke or how much tobacco do you use per day? At what age did you start using tobacco? Are there other tobacco smokers in the home?
- **Do you drink alcohol?** How much per day or week? At what age did you start?
- **What medications or drugs are you taking?** (Include prescription and non-prescription uses).
- **Has any one in the family worked with hazardous materials that they might have brought home** (e.g. pesticides, asbestos, lead)? If yes inquire about household members potentially exposed.)
2. Pediatric Patient (questions asked to parent or guardian)

**OCCUPATIONAL EXPOSURE:**

- What is your occupation and that of other household members?
- Describe your work and what hazards you are exposed (e.g. pesticides, solvents or other chemicals, dust, fumes, metals, fibers, radiation, biologic agents, noise, heat, cold vibration).

**ENVIRONMENTAL EXPOSURE HISTORY:**

- Are pesticides (e.g. bug or weed killers, flea and tick sprayers, collars, powders, or shampoos) used in your home or garden or on your pet?
- If pesticides used
- Is a licensed pesticides applicator involved?
- Are children involved allowed to play in areas recently treated with pesticide?
- Where the pesticides are stored?
- Is food handled properly? (e.g. washing of raw fruits and vegetables)?
- Has the patient ever lived near a facility which could have contaminated the surrounding area (e.g. mine, plant, smelter, and dumpsite)?
- Has the patient ever changed residence because of a health problem?
- Does the patient's drinking water come from a private well? City water supply, and/or grocery store?

**SYMPTOMS AND MEDICAL CONDITIONS**

- Does the timing of symptoms have any relationship to environment activities listed above?

- Has any other household member or nearby neighbor suffered similar health problem?
NON OCCUPATIONAL EXPOSURES POTENTIALLY RELATED TO ILLNESS OR INJURY

- Are there tobacco smokers in the home? If yes in what forms (cigarettes, pipe, cigar, chewing tobacco)?
- What medications or drugs is the patient taking? (Include prescription and non-prescription uses)

- Has anyone in the family worked with hazardous materials that they might have brought home (e.g. pesticides, asbestos lead)? (If yes, inquire about household members potentially exposed).

High risk patients, the exposure history should include specific questions about the agricultural work being done for example:

- Are pesticides being used at home or work?
- Were the fields wet when you were picking?
- Was any spraying going on while you were working in the field?
- Do you get sick during or after working in the fields?
Laundering Recommendations for Pesticide Handlers

1. Keep pesticide clothing separate from family cloths before and during laundering.

2. Pre-rinse or pre-soak clothing and discard rinse or soak water.

3. Use hot water to wash, cold to rinse.

4. Wash only a few items at a time. High water volume enhances residue removal, so do not overcrowd washer.

5. Use the highest water level setting, even for small loads.

6. Use the longest wash time cycle – at least 10 to 12 minutes.

7. Never use sudsaver feature.

8. Do another complete wash cycle before drying.

9. If the second washing does not remove stains or odor discard the clothes.

10. If possible, hang clothes to dry in the sun. Sun helps degrade some pesticides.

11. Before laundering family clothes run the washer through a complete cycle without clothes. Use hot water and detergent.

Other Information:

1. Ammonia is not effective in residue removal.

2. A three hour soak in chlorine bleach solution may help remove chlorpyrifos.

3. Fabric softness neither helps nor hinders residue removal.


5. Salt (1 cup per load) helps removal paraquat but not other pesticides.

6. Starch may help prevent pesticides from reaching the skin.
Messages to help farmworkers and their families reduce their exposure to pesticides.

❖ Wear appropriate protective work clothes.
❖ Do not enter recently sprayed fields.
❖ Wash hands frequently with soap and water.
❖ Drink 6-8 glasses of water a day.
❖ Remove boots and hats before entering home.
❖ Take Shower and put on clean clothes immediately after returning home from work.
❖ Wash work clothes after one wearing and separately from other family laundry.
❖ When picking up children from day care directly after work, use a clean blanket or towel to carry them.
❖ Do not let children play in fields or swim in irrigation ditches.
❖ Always wash fruits and vegetables before eating.
❖ Clean home, vehicles and pets regularly.
❖ Use and store pesticides safely.
❖ Use pesticides alternatives in the home and garden
❖ Protect against sprays drifting in to the yard from near by fields.
ANDHRA PRADESH

District Map
Area Wise (Research Area)

1. Ramachandrapuram
2. Cheemalapadu
3. Rudravaram
4. Kudapa
5. Reddy gudam
Different steps involved in using of different OP pesticides

Purchasing, Storage of OP pesticides:

Storage of OP pesticides
Purchasing of different OP pesticides

Storage of formulated mixture of OP pesticides before spraying
Aerial spraying of OP pesticides

Mixing and Spraying of OP pesticides in Agricultural farms
Mixing of OP pesticides

Different apparatus used in spraying of OP pesticides
Transport of OP pesticides for spraying
Aerial spraying of OP pesticides
Organophosphorous pesticides are used in different crops and their composition

<table>
<thead>
<tr>
<th>Crop</th>
<th>Crop Name in Telugu</th>
<th>Organophosphorous Formulations</th>
<th>Rate</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.5 ml.</td>
<td>Available in the market.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.0 ml.</td>
<td>Available in the market.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.0 ml.</td>
<td>Available in the market.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.0 ml.</td>
<td>Available in the market.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5 ml.</td>
<td>Available in the market.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.4 ml.</td>
<td>Available in the market.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.5 ml.</td>
<td>Available in the market.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5 ml.</td>
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</tr>
<tr>
<td></td>
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<td>0.75 ml.</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5 ml.</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.0 ml.</td>
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<tr>
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<td></td>
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<td>3.0 ml.</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3.0 ml.</td>
<td>Available in the market.</td>
</tr>
</tbody>
</table>

*For more information, contact Bayer CropScience.

Bayer CropScience

For more information, visit Bayer CropScience online or contact your local representative.
FARMS

PARRYSULFAN 35 EC
ENDOSULFAN 35% EC

<table>
<thead>
<tr>
<th>Crop</th>
<th>Dose (ml)</th>
<th>Area (ha)</th>
<th>Rate (litre/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato</td>
<td>500</td>
<td>0.1</td>
<td>5000</td>
</tr>
<tr>
<td>Cucumber</td>
<td>500</td>
<td>0.2</td>
<td>2500</td>
</tr>
<tr>
<td>Tomato</td>
<td>500</td>
<td>0.1</td>
<td>5000</td>
</tr>
<tr>
<td>Cucumber</td>
<td>500</td>
<td>0.2</td>
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</tr>
<tr>
<td>Tomato</td>
<td>500</td>
<td>0.1</td>
<td>5000</td>
</tr>
<tr>
<td>Cucumber</td>
<td>500</td>
<td>0.2</td>
<td>2500</td>
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</tbody>
</table>

Coromandel
1-2-10, Rallapalli, Andhra Pradesh-500 003.
A Novel Spectrophotometric Method for Determination of Propyl Gallic Acid by Oxidative Coupling with Orcinol

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2Department of Biochemistry, Acharya Nagarjuna University, Post Graduate center, Nuzvid-521 201.
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4Department of Biochemistry, Vananchal Dental College, Jarkhand, India.

(Received: 08 February 2008; accepted: 16 March 2008)

A simple, sensitive and reproducible spectrophotometric method is developed for the determination of propyl gallic acid. This method is based on oxidative coupling reaction between propyl gallic acid with orcinol in the presence of Hydrogen peroxide and enzyme horseradish peroxidase to produce colored chromogen (λmax at 550 nm). Results of analysis were validated statistically and by recovery studies. This method is successfully employed for the determination of propyl gallic acid in oils.

Key words: Propyl gallic acid, orcinol, Visible Spectrophotometry, Beer's Law.

Propyl gallic acid is “Propyl ester of gallic acid, n- propyl ester of 3,4,5-trihydroxy benzoic acid, propyl 3,4,5-trihydroxy benzoate” is an important naturally derived antioxidant. Propyl gallate is made from natural gallic acid, which is obtained by the hydrolysis of tannins from Tara pods. It is a fine, white to nearly white powder. It exhibits excellent antioxidant activity in food and vegetable oils, especially in combination with ascorbyl palmitate. Propyl gallate is mainly used as antioxidant additive in fats, oleaginous foods and medicinal preparations and to stabilize cosmetics, adhesives, and lubricants, food packaging materials. It is used to prevent rancidity in oily substances. Exploiting the various functional groups present in the above compounds, the authors have made attempts in this direction and succeeded in developing a spectrophotometric method for the determination of propyl gallic acid to produce colored chromogen (λmax at 550 nm).

EXPERIMENTAL

Instrumentation

Spectral and absorbance measurements are made with Systronics UV-Visible Double beam spectrophotometer model 2201.

Reagents

All the chemicals used were of analytical grade. All the solutions were freshly prepared with double distilled water. Freshly prepared solutions were always used. Aqueous solution of propyl gallic acid (0.1% w/v), orcinol (0.3 % w/v), hydrogen peroxide (0.01M), phosphate buffer (0.1
M, pH 7.0) and extracted enzyme Horseradish Peroxidase were used.

Standard and Sample solution of Gallic acid

About 100 mg of Propyl gallic acid was accurately weighed and dissolved in 100 ml of alcohol in a volumetric flask to make a solution of 1 mg/ml standard solution and further dilutions are made with the same solvent.

Extraction of the enzyme (Horseradish Peroxidase)

A turnip (Horseradish root) weighing 40 g was peeled, washed, and cut into 1" cubes. The sliced pieces were homogenized in 200 mL of buffer in a blender at high speed for 15 minutes. The extract is clarified by centrifugation (10,000 rpm/10 min.) and filtered through Whatman No. 1 filter paper. The extract for stability was stored in toluene for at least a week at 4°C. The extract was suitably diluted for further experimental analysis.

Assay Procedure

Into a series of 25 ml calibrated test tubes, 15 ml buffer (pH 7.0) solution, 2 ml of reagent (orcinol), 1 ml of hydrogen peroxide (0.01M) and 2 ml horse radish root solution (1:1 diluted) and aliquots of standard antioxidant solution, were added and made up to the mark with distilled water. The absorbance was measured after complete color formation at \( \lambda_{max} \) of 550 nm against reagent blank. The amount of antioxidant was computed from the calibration graph and the results were incorporated in Table 1. The proposed method is sensitive and accurate with reasonable precision and accuracy. The method could also be extended for the recovery propyl gallic acid in edible oils and fats.

RESULTS AND DISCUSSION

The optimum conditions for the color development was established by varying parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species. The following experiments were conducted for the purpose and the conditions so obtained were incorporated in Table 1. The absorbance's at corresponding series of varying one and fixing the other three parameters (pH, concentration of reagent and enzyme (HRP)/H\(_2\)O\(_2\) concentrations) containing in a total volume of 25 ml are measured against corresponding blank in each case. Performed recovery experiment and percent recovery values obtained are listed in Table 2. Recovery experiment indicated the absence of interference from the commonly encountered additives and excipients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{max} ) (nm)</td>
<td>550</td>
</tr>
<tr>
<td>Beer's law limit (µg/ 25 ml)</td>
<td>100 - 500</td>
</tr>
<tr>
<td>Sandell's Sensitivity (µg/cm(^2)/0.001 abs. unit)</td>
<td>0.0163</td>
</tr>
<tr>
<td>Molar absorptivity (Litre.mole(^{-1}).cm(^{-1}))</td>
<td>2.0162 \times 10(^{-4})</td>
</tr>
<tr>
<td>Optimum photometric range (µg/ 25 ml)</td>
<td>97 - 447</td>
</tr>
<tr>
<td>Time taken for Color development (Min)</td>
<td>5</td>
</tr>
<tr>
<td>Stability of Color (Min)</td>
<td>60</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oil</th>
<th>Quantity of propy gallic acid (µg)</th>
<th>% Recovery by Proposed method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coconut</td>
<td>10</td>
<td>98.4</td>
</tr>
<tr>
<td>Groundnut</td>
<td>97.2</td>
<td>98.5</td>
</tr>
</tbody>
</table>

Table 2. Recovery of Propyl gallic acid in various oils

The molar extinction coefficient, optimum photometric range and Sandell's sensitivity values of the proposed method were calculated and the results are incorporated in Table 1.

The proposed method is sensitive and accurate. The method has been extended for the recovery of propyl gallic acid in edible oils and fats. Thus the proposed method is simple and sensitive with reasonable precision and accuracy. This can be used for the routine determination of propyl gallic acid in quality control analysis.
ACKNOWLEDGEMENTS

The authors are grateful to Managements of Siddhartha Academy, Vijayawada, Acharya Nagarjuna University Post Graduate center, Nuzvid and Vananchal College, Jarkhand for their continuous support and encouragement and for providing the necessary facilities.

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Abstract

2- 35th National Conference of Association of Clinical Biochemists of India (35th ACBICON 2008)

Biologic Monitoring of metabolites of organophosphate pesticides in urine of children in an agricultural community

S.K.Rastogi* , P. V. V. Satyanarayan **, D. Ravishankar* *, Sachin tripathi*
*Indian institute of toxicology research **Department of Biochemistry, Acharya Nagarajuna University, Guntur (A.P.) India

The exposure of children to environmental toxicants has become the focus of increased public health concern over the last decade. The determination of pesticide residues or metabolites in biologic fluids of the exposed population has recently been the subject of many research studies. Exposure to organopesticides among children may be through dietary, respiratory and dermal routes. Children’s exposure to pesticides is potentially greater than that of adults. Studies reported by U.S. Environmental Protection Agency revealed that Children have a 12-time greater health risk than adult associated with the ingestion of dust and soil. Measurement of dialkyl phosphate (DAP) compounds in urine has been used to assess exposure to organophosphate pesticides in children living in rural agricultural settings in northern India.

We quantified DAP levels in serial samples of urine from 37 children, 6-10 year of age belonging to a neighboring village of Lucknow. The most commonly detected metabolite, dimethylthiophosphate (DMTP) was significantly higher in urine samples from children of the agricultural community relative to a reference group of children who lived in an urban community and whose parents did not work in agriculture. Fifty seven 10-14 year-old children who had Para occupational exposure to organophosphorus pesticides
as one of their parents where pesticides sprayer in the neighboring rural area of Lucknow (India) underwent biologic monitoring to evaluate urinary excretion of six alkyl phosphate that are metabolites of op pesticides. The collection of urine samples was accompanied by a questionnaire on lifestyle and dietary habits. The urine samples taken at Primary Health Center were analyzed for alkyl phosphates by gas chromatography with flame photometric detection. We found dimethylphosphate (DMP) and dimethylthiophosphate (DMTP) in detectable concentration in 67% and 76% samples respectively. The DMP values were geometric mean (GM) 239.8 and a range of 96.4-876.5 nmol/g creatinines while DMTP values were GM 197.6 and range of 60.7-1011.7 nmol/g creatinine. Other OP metabolites like diethyl phosphate (DEP) dimethyldithiophosphate (DMDTP) and diethylthiophosphate (DETP) concentration were less conspicuous. The etiological variables for urinary excretion of these metabolites in children could be domestic storage, involvement in manual mixing and handling of the pesticides prior to their spraying.
Abstract presented in conference


A study on oxidative stress in farming community exposed to Organophosphate pesticides

S.K.Rastogi* , P. V. V. Satyanarayan **, D. Ravishankar* *, Sachin tripathi*
*Indian institute of toxicology research**Department of Biochemistry, Acharya Nagarjuna University, Guntur (A.P.) India

Abstract

Pesticide poisoning is an important cause of morbidity and mortality in agricultural workers in developing countries. Every year there are 3 million cases of poisoning and 220,000 deaths; the majority of these poisonings and 99% of the resulting deaths occur in the third world. Lindane, malathion and propoxur are widely used organochlorine, organophosphate and carbamate pesticides, respectively. Undesirable side-effects that result from the indiscriminate use of these pesticides are widespread. These pesticides are known to disturb the biochemical and physiological functions of erythrocytes and lymphocytes. Despite existing knowledge following research, it cannot be predicted, to what extent a chronic exposure of pesticides will cause neurotoxicity in exposed group.

Free radicals play an important role in toxicity of pesticides and environmental chemicals. Pesticide chemicals may induce oxidative stress leading to generation of free radicals and alteration in antioxidants or oxygen free radical (OFR) scavenging enzyme system. Lipid peroxidation has been suggested as one of the molecular mechanisms involved in pesticide-induced toxicity. OFR enzymatic scavengers like superoxide dismutase (SOD), catalase (CAT), gamma-glutamyl transpeptidase (GGT), glutathione-S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR) etc., may protect the system from deleterious effect of OFRs. Further, lymphocyte membrane contain cholinergic receptors as well as GGT which play an important role in the metabolism of
xenobiotics like malathion. Erythrocytes and lymphocytes have a variety of redox systems among which glutathione (GSH) is important.

The study investigated oxidative stress, and derangement of the antioxidant defense system in blood samples obtained from farming community exposed to organophosphate pesticides. In addition, the activities of acetylcholine esterase and GGT, and level of GSH in lymphocytes following exposure to these pesticides were also evaluated.

The results indicated increased levels of SOD, catalase, Glutathione reductase (GR), Glutathione peroxidase (GPx), glutathione-s- transferase and GGT while GSH level was decreased. AchE activity also decreased in the exposed workers. The present results indicate that OFR scavenging enzymes were induced while combating oxidative stress. Increased lipid peroxidation with altered GSH and OFR scavenging enzymes in the blood are discussed with regard to oxidative stress as the susceptibility of erythrocytes and lymphocytes to oxidative stress due to exposure to OP pesticides is a function of overall balance between the extent of oxidative stress and the antioxidant defense capability.
A study on oxidative stress and antioxidant status of agricultural workers exposed to organophosphorus insecticides during spraying

S.K.Rastogi *. P. V. V. Satyanarayan **, D. Ravishankar* * and Sachin Tripathi
*Indian Institute of toxicology Research, Lucknow (U.P.) India ** Department of Biochemistry, Acharya Nagarajuna University, Guntur (A.P.) India

Abstract

Oxidative stress status and acetylcholinesterase (AChE) activity were studied in blood samples obtained from 61 agricultural workers engaged in spraying the organophosphorus insecticides in the mango plantation with a minimum work history of 1 year in the age range of 12-55. Controls were age matched unexposed workers never had any exposure to OP pesticides. They were evaluated for oxidative stress markers MDA (end product of lipid peroxidation), Reduced glutathione (GSH) and Acetylcholinesterase (AChE), butyrylcholinesterase (BChE) levels in blood. The results show marked inhibition of AChE and BCHE activity in sprayers as compared to the controls, increased blood MDA level, while GSH level, an antioxidant molecule was not significantly different between the both group. It is concluded that OP pesticides using by agricultural workers are exposed to more oxidative stress. The measurement of AChE, BCHE activity in agricultural workers who spraying OPs can be a good biomonitoring factor and is recommended to be performed in a regular manner.

Keywords:- Oxidative stress, AChE, OP pesticides
SK Rastogi*, D. Ravishanker**, Vishnumollkala Sridevi***, V.L.K Prakash**** and Peticam Lavudu****

*Indian Institute of Toxicology Research, Lucknow, India ** Acharya Nagarjuna University, Department of Biochemistry, Nagarjuna Nagar, Guntur, A.P India *** Applied Microbiology Department, Sri Padmavarthi Mahila University, Tirupathi, A.P, India **** Department of Biotechnology, Padmbhushan Dr. B.V Raju Institute of Computer Education, Bhimavaram, West Godavari District, A.P India

Abstract
A cross sectional study was conducted on 90 male agricultural workers (39 farm workers and 51 OP pesticide sprayers) to investigate the effects of acute and chronic OP pesticide exposure on the urinary levels of 8-OHdG and to compare the levels observed in the exposed workers with that obtained in the referent group. The study revealed significantly higher urinary levels of 8-OHdG in the farm workers as well as in the pesticide sprayers in comparison to the observed value in the control group thereby indicating that 8-OHdG is a sensitive and reliable biomarker of oxidative stress induced by occupational and non occupational exposure to OP pesticides. Based on the findings the study recommends the use of 8-OHdG evaluation in the target populations which will be helpful to assess the risk of genotoxicity caused due to oxidative stress in pesticide workers.

Keywords: Reactive oxygen species (ROS), Malondialdehyde (MDA), Organophosphate Pesticides and 8-Hydroxydeoxy guanosine (8-OHdG).
Introduction

Free radicals and other reactive species are constantly generated in vivo and cause oxidative damage to biomolecules, a process held in check only by the existence of multiple antioxidant and repair systems as well as the replacement of damaged nucleic acids, proteins and lipids. DNA is probably the most biologically significant target of oxidative attack and it is widely thought that continuous oxidative damage to DNA is a significant contributor to the age related development of the major cancers. Among numerous types of oxidative DNA damage the formation of 8-Hydroxydeoxyguanosine (8-OHdG) is a sensitive marker of oxidative stress. 8-OHdG, one of the oxidative DNA damage by products, is physiologically formed and enhanced by chemical carcinogens. Chronic exposure to OP pesticides is implicated in many health conditions that result from the induction of oxidative stress, including cytogenetic damage. Most widely used OP pesticides are anthropometric chemicals released into the environment for controlling agricultural pests. There are about 15000 individual compounds and 35000 formulations in use as agricultural pesticides. Though beneficial in their action, these toxicities account for a significant risk of occupational toxicity due to chronic exposure. At the cellular level, pesticides have been reported to generate ROS, which catalyze increased lipid per oxidation. ROS are removed by the endogenous antioxidant enzymes such as SOD, GSH, Catalase and other peroxidases.

One of the oxidized products of nucleic acids, 8-OHdG is a sensitive bio marker of oxidative stress. The substance, 8-OHdG is an adduct formed as a result of reaction between ROS and DNA. It establishes the link between intra cellular ROS accumulation and genotoxicity. 8-OHdG if allowed to accumulate can penetrate through the DNA replication process and can retard the DNA repair mechanism.
Aim

The study was proposed to evaluate the levels of 8-OHdG in spot urinary samples in the exposed OP pesticide sprayers and farm workers engaged in mango orchards at Mal and Malihabad areas adjoining Lucknow, UP and to compare the findings obtained in these groups with that recorded in the control group belonging to similar socioeconomic status having similar rural background but had no past or current exposure to OP pesticides.

Materials and Methods

In a cross sectional study 51 male pesticide sprayers and 39 farm workers belonging to the age group of 18 to 47 years having exposure which ranged from 3 years to 12 years in duration. While selecting the subjects the care was taken that they were actively farming, wherein they mixed and sprayed the OP pesticides as well as lived in the vicinity of the farms. Only those who had reported good health without any chronic health conditions were selected for the study. The referents (n=31) were selected on the criteria that these participants were never exposed to pesticides at any time even at home. The background information of all participants included smoking habits, years of smoking, types of OP pesticides used in the past and those currently in the farm, use of any safety measures such as protective clothing, nasal masks and hand gloves. None of the farm workers on clinical examination showed sub clinical symptoms associated with occupational exposure to OP pesticides. This study was conducted during the growing season (January, 2008 to May 2008). The survey questionnaire administered at the start of the study showed that the farm workers were using a combination of the following pesticides. The most commonly used OP pesticides were Endosulfan, Chlor pyriphos, Diazinon, Dimethoate etc.
Spot urine samples were collected at the end of the work day. Urine samples collected from each participant in sterile tubes and were stored at -20 degree centigrade till analysed. For analysis, each sample was brought to room temperature centrifuged at 1200g per 10 units and the 5 ml of supernatant was pooled. Concentration of 8-OHdG were analysed using Enzyme Linked Immunosorbent Assay (ELISA) kit.

**Statistical Analysis**

The data for the two groups were compared (Farm workers and controls) by the Tukey’s test and significant differences between the means were determined at $P < 0.05$.

**Results**

Table 1 shows the demographic characteristics of the exposed and the control groups. The controls and the exposed group did not show significant differences in their mean ages. Similarly the smoking profile of the controls and the exposed groups did not differ significantly. However the farm workers and the sprayers showed greater prevalence of alcohol consumption. The mean exposure to OP pesticides was similar in the farm workers and pesticide sprayers.

The levels of 8-OHdG in the urine samples of participants in both the groups are shown in table 2. A statistically significant difference at $P<0.05$ was observed in the levels of urinary 8-OHdG values between the exposed and the controls was observed. The urinary levels of 8-OHdG were significantly higher in the farm workers and pesticide sprayers in contrast to the level observed in the control group. However, the mean values observed in the case of farm workers and pesticide sprayers showed no significant differences.

When the data was analysed in the exposed group in relation to duration of exposure it was found that both the farm workers and sprayers who were exposed to OP pesticides for less then 5 years showed the maximum mean values of 8-OHdG in comparison to those exposed for more then 10 years (Table 3).
In our study we did not find any difference in the mean values of 8-OHdG between the smoking and non-smoking exposed group, thereby indicating that smoking in particular did not appear to affect the levels of 8-OHdG in urinary samples (Table 4).

Discussion

The present results indicate that there is statistically significant differences in the urinary levels of 8-OHdG in the exposed workers and the control groups. Thereby indicating oxidative stress and DNA damage and that the amount of DNA damage correlated with the extent of pesticide exposure. Some studies have suggested that oxidative stress and DNA damage are common mechanisms by which pesticide disrupt the function of human cells. Oxidative DNA damage reportedly plays an important role in a number of pathological conditions including carcinogenesis. However few epidemiological studies have reported the usefulness of measuring biomarkers of oxidative stress (8OHdG) and DNA damage. Lagorio and colleagues reported a dose response effect between occupational exposure to Benzene and urinary levels of 8-OHdG. In our study we did not find the influence of smoking and consumption of alcohol on the levels of 8-OHdG. Loft and Poulsen (1996) reported the correlation between body mass index and the levels of 8-OHdG. They observed that leanness is reported to be associated with increased excretion of 8-OHdG possibly due to the influence of a higher metabolic rate. We did not obtain body mass index in this study but it is possible that the high excretion associated with farm work could have contributed to higher urinary levels of 8-OHdG among agricultural workers. Similarly the role of diet is also set to influence the levels of excretion of 8-OHdG but in our study we fail to observe any difference between the levels of urinary 8-OHdG between the vegetarian and non-vegetarian exposed workers. We also did not find an association between self-reported alcohol consumption among the individuals participating in this study.
Both OP pesticide sprayers and farm workers had higher levels of 8-OHdG than controls. Previous studies that used 8-OHdG as a marker of oxidative damage used a 24-H urine collection but in our study only spot urines were feasible as spot urines contain adequate levels of 8-OHdG for measurement in the exposed workers. However, recent studies indicate that urinary levels of 8-OHdG can vary over a 24-H period when measured for consecutive days. Therefore we suggest that single values of 8-OHdG should be considered with caution, and when spot urines are used. Oxidative DNA lesions generally appear within hours after exposure. Mutagenic chemicals and their persistence may be brief as indicated by the diminished mutagenicity within 24-H or less after exposure. It may be that urinary levels of 8-OHdG reflect short-term exposures compared to the other markers examined in the serum (MDA) of agricultural workers. Thereby reflecting more current exposures to OP pesticides.

In summary, several factors could be responsible for the overall increase in urinary 8-OHdG concentrations observed in the exposed workers in this study. This type of study requires recruitment of farm workers and pesticide sprayers who have continuous exposure to pesticides, longer duration of exposure to pesticides.

Conclusion

The substance 8-OHdG is an adduct formed as a result of reaction between ROS and DNA. It establishes a link between intracellular ROS accumulation and genotoxicity. In view of this regular bio monitoring studies in target human populations are imperative and necessary due to frequent changes in pesticide formulations and introduction of newer pesticides.
References


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11) Lagorio S, Tagesson C, Forstiere F et.al; Exposure to benzene and urinary concentrations of 8-OHdG, A biological marker of oxidative damage to DNA. Occup Environ Med. 51, 739-743, 1994.


14) Panemangalore M; Dowla HA; Byers ME. Occupational exposure to agricultural chemicals. Effect on the activities of some enzymes in the blood of farm workers. Int Arch Occup Environ Hlth. 72, 84-88, 1999.
TABLE – 1
Characteristics of the Agricultural Workers and Rural Control Participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Farm workers</th>
<th>Pesticide sprayers</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of Participants</td>
<td>39</td>
<td>51</td>
<td>31</td>
</tr>
<tr>
<td>Average age (Years)</td>
<td>27.6±1.9</td>
<td>29.4±1.6</td>
<td>26.2±2.2</td>
</tr>
<tr>
<td>Period of Exposure</td>
<td>15.2±2.9</td>
<td>17.6±2.1</td>
<td>-----</td>
</tr>
<tr>
<td>Use of safety equipments</td>
<td>43.5</td>
<td>41.7</td>
<td>-----</td>
</tr>
<tr>
<td>Smoking prevalence (%)</td>
<td>53.8</td>
<td>52.9</td>
<td>58.0</td>
</tr>
<tr>
<td>Duration of smoking (years)</td>
<td>10.2±3.6</td>
<td>12.4±2.6</td>
<td>10.4±2.2</td>
</tr>
<tr>
<td>Bidis smoked/day</td>
<td>12 - 15</td>
<td>10 - 15</td>
<td>10 - 15</td>
</tr>
<tr>
<td>Alcohol consumption (ml)</td>
<td>500 - 700</td>
<td>350 - 500</td>
<td>400 - 700</td>
</tr>
</tbody>
</table>

TABLE 2
The Overall Mean Values of Urinary 8-OHdG (umol/molcreatinine) in Exposed Group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean±SE of urinary 8-OHdG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm Workers (n=39)</td>
<td>0.538±0.034</td>
</tr>
<tr>
<td>Pesticide Sprayers (n=51)</td>
<td>0.47±0.106</td>
</tr>
<tr>
<td>Controls (n=31)</td>
<td>-----</td>
</tr>
</tbody>
</table>
TABLE 3.

The Urinary Levels of 8-OHdG in Relation to Duration of Exposure

<table>
<thead>
<tr>
<th>Exposed Groups</th>
<th>5 to 10 years</th>
<th>11 to 15 years</th>
<th>&gt;15 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm Workers</td>
<td>0.489±0.150</td>
<td>0.363±0.132</td>
<td>0.255±0.071</td>
</tr>
<tr>
<td>Sprayers</td>
<td>0.522±0.091</td>
<td>0.488±0.087</td>
<td>0.375±0.011</td>
</tr>
</tbody>
</table>

TABLE 4

The Urinary Levels of 8-OHdG in relation to smoking habits

<table>
<thead>
<tr>
<th>Exposed groups</th>
<th>Mean±SE of 8-OHdG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm workers (Smokers-21)</td>
<td>0.363±0.050</td>
</tr>
<tr>
<td>Farm Workers (Non – Smokers – 18)</td>
<td>0.375±0.030</td>
</tr>
<tr>
<td>Pesticide Sprayers (Smokers – 27)</td>
<td>0.422±0.087</td>
</tr>
<tr>
<td>Pesticide Sprayers (Non - smokers – 24)</td>
<td>0.387±0.109</td>
</tr>
</tbody>
</table>
A study of the effect of occupational organophosphate exposure on neuropsychological functions of pesticide sprayers.

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ABSTRACT

Organophosphate compounds are extensively used as pesticides and industrial chemicals. They are primarily neuro toxic and produce well defined muscarinic, nicotinic and central nervous system effects by means of inhibition of acetyl cholinesterase. In view of their high acute toxicity and wide spread use, the effects of organophosphates assume great importance. The neurophysiological effects of organophosphate pesticides following repeated occupational exposure need to be investigated particularly in our country where the exposure conditions are quite unsatisfactory. Since estimation of acetyl cholinesterase (AchE) employed for monitoring organophosphate exposure has wide range of variation and a number of physiological factors affecting its level. This test was not found to be very useful in monitoring the agricultural workers and therefore the evaluation of neuropsychological functions gains importance. In this study an attempt has been made to evaluate the neurological effects following occupational OP pesticide exposure using objective and non-invasive neuropsychological techniques.
31 pesticide sprayers engaged in regular spraying of methyl and ethyl OP pesticides were evaluated along with a control group comprised 19 non-farming workers matched for age, sex, socio economic and nutritional status were also tested for comparison sake. The neurophysiological test included maximal motor conduction velocity of median and peroneal nerve, sensory conduction velocity of median and sural nerve, F response. Motor nerve conduction velocity of medial nerve (elbow to wrist segment) was measured by stimulating the median nerve supra maximally at the wrist between the tendon of flexor carpi radialis and palmaris longus. Sensory conduction velocity of the median nerve was measured orthodromically. Surface stimulation was performed by ring electrodes, the active electrode being placed at the proximal and the reference electrode at the distal phalangeal joint. The results indicated significantly reduced values of motor and sensory conduction velocity and F response in the pesticide sprayers. The median NCV was found to be 50.09 + 4.69 m/s in comparison to 58.91+5.06 m/s observed in the case of the unexposed control group. Similarly the peroneal NCV was also significantly affected in the pesticide sprayers (40.22+4.69 m/s) in comparison to 49.69+3.15m/s observed in the controls. Similarly the terminal latency was also decreased significantly in the exposed group. However the F response did not defer statistically between the exposed and the control groups. Our findings based on the neurophysiological tests suggests disturbances in the neurological functions in the organophosphate sprayers which was reflected in the significant reductions in median and peroneal sensory and motor conduction thereby indicating occurrence of peripheral nerve dysfunction in the pesticide sprayers.