

**COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT****REVIEW OF GOVERNMENT FUNDED RESEARCH: PAPER ON ORGANOPHOSPHATES AND DNA DAMAGE****BACKGROUND:**

1) In 1999, the COT published a report on organophosphates (OP's) which considered whether *prolonged or repeated low level exposure to organophosphates, or acute exposures to organophosphates at levels insufficient to cause overt toxicity, can cause long-term adverse health effects.*<sup>1</sup> In the report, COT had drawn conclusions from the available data and made recommendations for further research to address outstanding issues which relate to potential chronic ill health (such as neuropsychiatric, neuropsychological effects and evidence for the occurrence of 'sheep dippers flu'). The research has been funded jointly by a number of Government Departments with the Veterinary Medicines Directorate taking a coordinating role. The COT have considered research projects as they have been completed. The most recent consideration was in September 2009. (<http://cot.food.gov.uk/pdfs/tox200926.pdf>).

**INTRODUCTION**

2. One study, conducted as part of the Government funded research was research project VM02301, which examined evidence for genotoxic effects of organophosphate (OP) pesticide exposure in horticulture workers. Part of this study has recently been published as : Atherton, K.M, Williams, F.M. et al (2009) DNA damage in horticultural farmers: a pilot study showing an association with organophosphate pesticide exposure. *Biomarkers* **14(7)** 443-451 (attached as Annex 1). The COM are asked to consider this report and the questions in paragraph 14 below.

3. The principle overall project aims were to investigate whether there is a causal relationship between chronic OP exposure and DNA damage. The project reviews studies in animals *in vivo*, *in vitro* studies to investigate mechanisms and reports results of a bio-monitoring investigation which aimed to address the hypotheses that OPs can cause DNA damage to man following low-level chronic exposure.

4. It is noteworthy that the COM previously undertook detailed reviews of the published literature on the evidence for genotoxicity of pesticides in pesticide applicators and on factors influencing the background incidence of

genotoxicity biomarkers. Statements were written and the assessments published in peer review journals <sup>2,3</sup>

<http://www.iacom.org.uk/statements/documents/PesticideApplicators.pdf>

<http://www.iacom.org.uk/statements/documents/pbl0603.pdf>

[The relevant documents have been appended as Annex 2.]

#### **PUBLICATION SUMMARY:**

5. The study investigated DNA damage in a cohort of horticultural farmers from Spain who had been exposed to OP pesticides. In the Spanish cohort, 17 male horticultural workers (35.5 ±8.2 years of age) who had worked with pesticides for at least 2 years were recruited. Seven healthy men (26.0±4.8 years of age) from the same area, not occupationally exposed to OP's were included as controls (3 smokers, 4 non-smokers). The study design included 32 non-smoking men (33.5±9.0 year of age) from the UK who acted as controls providing information on background DNA damage as a consequence of typical low level exposure to environmental genotoxins. Five unexposed UK female subjects were also assessed (41.8±14.4 years of age).

6. Venous blood and urine were collected from the farmers in July and November and the following parameters were measured:

- DNA damage in peripheral lymphocytes using the Comet assay. DNA damage was expressed as olive tail moment (OTM) and also as a DNA damage score in which cells were classed as damaged 1-4, and when 100 cells were assessed the score range was 0-400.
- Intra-individual variation was measured in the UK females - DNA damage
- Urinary analysis of dialkylphosphate metabolites (DAP) as an exposure measurement specific to OP's. Analytical measurement range was 0.6-1.1 ng/mL.
- Acetylcholinesterase (AChE) activity in blood
- Butylcholinesterase (BuChE) activity in blood
- 8-hydroxy-2'-deoxyguanosine (8OHdG) in urine as a measure of oxidative stress.

#### **RESULTS**

##### **DNA damage:**

7. The intra-individual extent of DNA damage in the 5 unexposed females (mean and SD of measurement on 3 separate occasions), OTM values of 1.02 ± 0.741, 0.977±0.311, 1.55±0.067, 0.866±0.079, 1.69±1.02. DNA damage scores were 33±23, 16±3.5, 20±4, 8.0±0.1, 51±43.

Comparison of farm workers to Spanish and UK controls.

DNA damage scores (means) of 79.2±26.7, 50.0±15.1, 43.5±23.4 respectively. Statistically significant at p<0.05 and p<0.001 for Spanish and UK controls respectively.

### **Exposure to OP's as a measurement of Blood AChE:**

8. Mean activities for farmers, Spanish and UK controls respectively were  $3.11\pm 0.39$ ,  $4.20\pm 0.43$ ,  $4.26\pm 0.44$   $\mu\text{mol/mL/min}$ . This represents an approximate 30% reduction in farmers, the authors state that this indicates a moderate exposure. AChE was lower for farmers in July  $2.86\pm 0.20$  compared to November  $3.35\pm 0.39$ . There were no differences in BuChE levels between controls and farmers.

### **Urinary DAP levels of dimethyl and diethyl containing urinary DAP's:**

9. In farmers mean DMP+DMTP and DEP+DETP levels were  $0.054\pm 0.01$  and  $0.150\pm 0.035$   $\mu\text{mol/mmol creatinine}$  respectively. In the Spanish controls, levels were at the limit of detection (0.6-1.1  $\text{ng/mL}$ ). Sampling in July and November yielded different results for the DEP+DETP levels  $0.232\pm 0.19$   $\mu\text{mol/mmol creatinine}$  in July compared to  $0.07\pm 0.05$  in November, whereas the variation was not noted for DMP +DMTP  $0.06\pm 0.03$  compared to  $0.05\pm 0.03$ .

10. It is claimed that there is a substantial relationship between OP exposure and DNA damage ( $p < 0.02$ ). DNA damage was higher in July compared with November correlating with higher DAP and lower AChE. In addition, there is an apparent relationship with oxidative stress, as indicated by urinary 8OHdG levels. This was higher in farmers ( $1.17\pm 0.697$   $\text{pmol/mmol creatinine}$ ) compared to the Spanish controls ( $0.515\pm 0.314$ ). There is also a positive association between DAP and 8OHdG

11. In the discussion, the authors emphasized the importance of the relationship between the markers for OP exposure and DNA damage. This includes the differences between the July and November sampling times. However the results are given as averages for these two time-points combined despite the fact that there are assumed difference in exposure between July and November sampling time points. Furthermore, there is no discussion of the pattern of pesticide use at these times. The authors comment that the cohorts are small. This is particularly notable for the different sampling times; in July there were only 4 samples taken from farmers assessed for DNA damage.

12. Discussion of confounding factors is limited to smoking and age. It was stated that smokers had higher oxidative stress and DNA damage levels. A number of factors were highlighted in the COM review of factors which affect the incidence of genotoxicity biomarkers in peripheral blood lymphocytes<sup>3</sup>. Risk factors with strong/sufficient evidence for an association with genotoxicity were: age, gender, micronutrient status and smoking status.

13. The authors suggest that exposure to non-OP pesticides, carbendazim in particular, would be unlikely to have influenced the relationship seen

between OP exposure and DNA damage. However, in the COM review of pesticide workers it was suggested that there may be an association between benzimidazole use and genotoxicity <sup>2</sup>.

#### **Questions to Committee Members:**

14. Members are asked to evaluate this paper, taking into consideration the previous COM reviews of pesticide applicator exposure and risk factors for genotoxicity biomarkers.

- Members are asked to comment on the overall study design and the presentation of the results?
- What are Members opinion on the statistical methods used (correlation analysis)?
- What are Members opinion of the interpretation of the results and the conclusions?

#### **References:**

1. The COT report on organophosphates. November 1999.  
<http://cot.food.gov.uk/cotreports/cotwgreports/organophosphates>
2. Bull, S, Fletcher, K, Boobis, A, Battershill, J (2006) Evidence for genotoxicity of pesticides in pesticide applicators : a review *Mutagenesis* **21** 93-103
3. Battershill, J, Burnett, K, Bull, S (2008) Factors affecting the incidence of genotoxicity biomarkers in peripheral blood lymphocytes: impact on design of biomonitoring studies. *Mutagenesis* **23** 423-37