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DRAFT

MUT/05/12

COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT.

Biomonitoring studies of genotoxicity in pesticide applicators.

Discussion paper on evaluation of positive studies and control data.

Introduction

1. The Medical and Toxicology Panel of the Advisory Committee on Pesticides has asked the COM for advice on biomonitoring studies of genotoxicity in pesticide applicators. The DH Toxicology Unit in collaboration with the secretariat drafted an initial overview of studies from the EU. (MUT/04/19). Further areas identified for additional consideration at the February 2005 meeting included a discussion on mutagenicity/genotoxicity criteria for the identification of acceptable studies, the variance on control data, and the potential exposure to category 2 or 3 mutagens in some of the studies which had reported positive findings. (In essence chemicals which are *in-vivo* mutagens in experimental animals (category 3) and those also shown to be *in-vivo* germ cell mutagens in animals (category 2) A paper was subsequently drafted following the February 2005 COM meeting in liaison with one member of the COM, and circulated to members to reach agreement on criteria for study identification. Some initial comments on data evaluation were also made in this paper (MUT/05/10).
2. Information in paper MUT/05/10 was used to identify 24 selected studies which were submitted for an epidemiological overview.
3. A discussion of two further papers prepared by the DH Toxicology Unit in collaboration with the secretariat is given below. These two papers present information on possible approaches to evaluation of the results of biomonitoring studies in particular the evidence for a positive response (Annex 1) and the evaluation of studies where information on potential exposure has been provided together with a comparison of pesticide usage data and category 2 and 3 mutagens (Annex 2). All of the pesticide applicators would have used a number of pesticides products and thus been exposed to a mixture of pesticidal active ingredients. However for most studies the only available information is a listing of active ingredients used with some information on frequency of use in a number of the reports. Biomonitoring of exposure was only reported a very limited number of accounts (e.g. Garry et al 2001, EHP, 109, 495-500, 2001 for 2,4-D and Mustonen et al, 1986, Mutagenesis, 1, 241-245, 1986) and in these reports this accounted for only a proportion of the total pesticide exposures.

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Criteria for evaluation of studies (CC/05/10 and Annex 1 to this paper)

4. COM members noted the relatively small increase in indices of mutagenicity in many of the studies considered at the October 2004 and February 2005 meetings and asked for an evaluation of control data and consideration of criteria for a positive response. Some initial ideas were presented in MUT/05/10 which focused on one possible approach (an upper bound estimate for the control CA or MN range). The conclusions reached were based on the 24 studies selected for epidemiological overview. The mean, median, 95% confidence intervals, range and fold difference between lowest and highest data for these studies were reported in table 1 of MUT/05/10 (and also in table 2 of Annex 2 to this paper). Essentially there was an approximate 16 fold variance in control MN or CA data in these studies. Modelling of the data from the 24 selected studies suggested that MN data were normally distributed whilst there was evidence for a skewed distribution of CA (More information on the distribution of data is presented in Annex 1 to this paper). The large variance in control CA and MN data from the selected 24 studies reflecting in part methodological differences between studies regarding CA and MN data, suggests that it is not possible to define a historical control range which could be useful in interpreting the data from biomonitoring studies of genotoxicity in pesticide applicators.

5. The observation made at the February 2005 COM meeting that the magnitude of CA and MN changes reported appeared small in many studies prompted a further evaluation of these data. Information on the magnitude of CA or MN responses in biomonitoring studies of individuals either occupationally exposed or given doses of cytotoxic medicines used cancer therapy was obtained. The chemicals studies included cyclophosphamide, mephalan and etoposide. The mean fold increase in nurses exposed to cytotoxic medicines was 1.8 (range 1.5-2.2), in patients undergoing treatment was 2.1 (range 1.5-2.7) and biomonitoring studies of pesticide applicators was 1.7 (range 1.2-5.0) (see Annex 1). Although there would have been differences between the various groups compared (i.e. nurses, patients and pesticide applicators) regarding the chemicals to which they were exposed, the potency of any mutagens to which they were exposed, the extent of exposure, the timing of exposure in relation to sampling and the methods of cytogenetic evaluation between these groups, the small statistically significant increases in mutagenic indices in biomonitoring studies of pesticide applicators should be considered as providing evidence of exposure to mutagens. It is also possible that potential confounding factors exist which haven't been adequately controlled for, but these would have to exist in quite different studies and study populations. This conclusion essentially agrees with the advice on interpretation of results reached by Albertini (Mutation Research, 463, 111-172, 2000) who recommended that statistical significance and reproducibility supported by evidence for a dose-response were the key criteria for each assay. Albertini notes that dose-response evaluation can be complex for some end points. Thus the relationship between frequency MN in peripheral blood lymphocytes

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and exposure may be related to long-term exposure to mutagens which may be difficult to estimate or may not have been assessed appropriately in the studies under consideration

6. .. It might be possible to give greater weight to studies which reported additional validation information, e.g. an effect of smoking in controls, or protective effects of barrier clothing). Overall it is suggested that it is not possible to identify a minimum fold increase which would be relevant for the COM evaluation of pesticide applicators from the available information.

Evaluation of available information on exposure and relevance for UK situation.

7. An evaluation of the limited information on potential exposures is outlined in Annex 2. This comprised predominantly of listings of pesticide active ingredients documented in individual biomonitoring study reports which has been compared with published information on the classification status for mutagenicity under the Dangerous Substances Directive EC/67/548. The limitation to this approach is that information on pesticides which were used was only provided for 14 out of the 24 selected positive studies (11 with reported positive results, 3 with negative results). It is unclear whether the information reported identified all pesticides which had been used and how the information presented related to actual exposures close to the time when biological samples were obtained. There were no biomonitoring studies retrieved which had been undertaken in UK pesticide applicators and thus the data from the selected 24 studies was compared with information on usage trends in the U.K. This latter approach might help to identify potential exposure groups in the U.K. which might be suited for further research. The information on classified mutagens has been considered first in this covering paper.

7. There were a number of pesticides classified as category 2 or 3 mutagens identified in the selected studies of pesticide applicators which included fenthion, monocrotophos, benomyl, carbendazim, thiophanate methyl, DNOC, and diuron. {There are no current UK approvals for fenthion, monocrotophos, benomyl, and DNOC for use in agricultural/ horticulture, and floriculture uses} It is difficult to draw any conclusions from this information other than the possibility that exposure to known mutagens might explain the results of some of these biomonitoring studies. However, it is noted that 5/11 or more of studies with positive mutagenicity also documented potential exposure to a number of benzimidazole fungicides. It is suggested that a preponderance of certain active ingredients in the selected positive studies which are also approved for use in the U.K. (such as benzimidazoles carbendazim and thiophanate-methyl) could aid in the identification of possible active ingredients for a biomonitoring study in the U.K. It is noted that diuron was cited in one positive and one negative study. There would appear to be less convincing evidence regarding diuron. A review of the mutagenicity of diuron is outside the remit of the current COM review.

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8. Members will recall that the COM has accepted that a threshold approach to risk assessment can be undertaken for exposure to benzimidazole fungicides in view of the mutagenic mode of action involving a thresholded step (reversible binding and subsequent effects on microtubule formation and function). The finding by Bolognesi et al (Mutation Research, 557, 109-117, 2004) of a higher percentage of C+ micronuclei (centromere positive) in subjects using benzimidazolic pesticides would support the arguments outlined in this paper that these compounds are potentially associated with the positive findings reported in some of the reviewed biomonitoring studies. (This paper is being subject to the epidemiological overview currently being undertaken for the COM)

9. A separate approach was also undertaken to aid in the identification of uses in the U.K. which equated to Floriculturalists/green house workers and agriculturalists/farmers in the published studies. Publicly available information from the Pesticide Usage Survey reports was used to define uses and particular crops which could be considered as equivalent to the occupational categories cited in the published reports. The overall conclusion reached was that certain active ingredients cited in positive biomonitoring studies had increased in use in the U.K in practices which considered to equate to the floricultural and agricultural practices cited in the biomonitoring studies, for example carbendazim and bifenthrin respectively. (COM members will be aware of the evidence regarding mutagenicity of carbendazim and thiophanate-methyl which have been considered previously by COM. Regarding bifenthrin, the WHO JMPR has concluded in 1992 that there was no evidence from a substantive range of mutagenicity tests to indicate the bifenthrin is an *in-vivo* mutagen).

10. A comparison of the information on the category 2 or 3 mutagens listed in positive biomonitoring reports and the information from the Pesticide Usage Survey evaluation suggests that the use of carbendazim, methylbromide, and diuron have all increased over the past decade. It is beyond the scope of the COM review to make any statements regarding the regulatory review status of individual pesticide active ingredients (which is a matter for the Advisory Committee on Pesticides). These active ingredients would only be appropriate for further consideration if COM are able to conclude that the evidence suggests exposure to these active ingredients could have been implicated in some of the positive results reported and there is evidence for exposure in UK practices. The action for COM might be to highlight any particular pesticide active ingredients for further review in its statement. In this respect the best evidence appears to be for the benzimidazoles, carbendazim and thiophanate-methyl whilst there is comparatively less evidence regarding diuron.

Conclusions/questions for COM.

11. Members are asked to consider the most appropriate approach to evaluating positive data from biomonitoring studies in pesticide applicators. Do Members agree that the most appropriate approach is to consider statistically significant results for adequately conducted studies?

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12. Are members able to draw any conclusions on the available data on possible exposures in the positive studies? Does the citation of certain active ingredients, which are also classified for mutagenicity under EC/67/548, in a number (e.g 5 or more) of the 11 positive published studies such as benzimidazole fungicides suggest the need for further consideration of these chemicals, possibly through an appropriate U.K. based study.?

13. Does the combination of evidence for increasing use (from publicly available information from the Pesticide Usage reports) and citation in published biomonitoring studies reporting positive results, particularly as classified mutagens strengthen the evidence regarding the need for further review of benzimidazole active ingredients used in the U.K possibly through appropriate biomonitoring studies in the U.K.

14. Alternatively do members consider that the available information is too weak to draw any conclusions on possible exposures which might be associated with positive results in the biomonitoring studies? If so this would limit any conclusions which could be reached with regard to particular active ingredients.

15. Advice on the epidemiological overview of the selected studies is being prepared for the COM. Given this information is available and assessed at the May 2005 COM meeting, are members able to draw tentative conclusions? The secretariat propose that sufficient information is now available to begin to draft an initial working paper.

Secretariat April 2005

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ANNEX 1 TO MUT/05/12

Positive response in cytogenetic tests in biomonitoring studies in pesticide applicators

1. As part of the 'Genotoxicity in pesticide applicators' project, an attempt was made to ascertain the magnitude of increase in cytogenetic endpoints that would be deemed a positive result.

Guidelines

2. In the IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans, Albertini states that the study protocol should be specified in advance and should include the size of effects that would be considered biologically important (Albertini, *et al.*, 2000). In addition, internal quality assurance procedures should be described i.e. the expected differences between smokers and non-smokers, or young and old subjects could be used as a positive control. Moreover, the OECD guideline 473 (*In vitro* Mammalian Chromosome Aberration Test) states that positive controls should give a detectable increase over background that demonstrate the sensitivity of the test system.

3. From the 24 studies selected for further evaluation, none stated the magnitude of increase that would be considered biologically relevant in their study.

Magnitude of response following exposure to IARC class 1 mutagenic compounds: Relevance of data for assessing pesticide biomonitoring studies

4. In order to investigate the magnitude of increase in CA and MN that could be achieved in biomonitoring studies with known mutagenic agents, patients treated with, or nurses occupationally exposed to cytotoxic drugs that had been identified as IARC category 1 mutagens (carcinogenic in humans), such as cyclophosphamide, melphalan and etoposide were studied. The patients were regularly treated (usually every 4-6 weeks) with the cytotoxic drug as part of the therapeutic regime, usually by intravenous infusion, whereas nurses were exposed almost daily during the preparation and administration of the drugs.

5. Table 1 and figure 1 show the fold increase in such biomonitoring studies with nurses or patients. Data show that a mean fold increase of 1.8 (range 1.5 to 2.2-fold increase) was achieved in nurses following occupational exposure compared to non-exposed workers. In patients treated with such drugs, the mean fold increase was 2.1 (range 1.5 to 2.7-fold increase) compared to non-treated patients.

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6. In comparison to the biomonitoring studies with known mutagenic drugs, studies in workers undergoing pesticide exposure showed an overall fold increase in cytogenetic endpoints of 1.7 (range 0.8-5; figure 1).

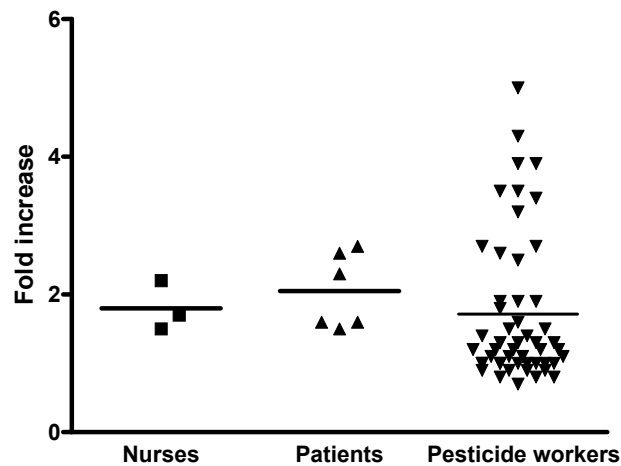


Figure 1. Magnitude of response in nurses and patients exposed to cytotoxic agents and workers exposed to pesticides

Cytogenetic endpoints

7. Both CA and MN were measured in the biomonitoring studies with pesticide workers and with patients or nurses. Most experimental protocols used for either CA or MN analyses were similar. However, in studies with nurses or patients, a longer incubation time was used in the MN assay whereas in the selected studies of pesticide workers, most studies used a shorter incubation time or supported MN frequency data with proliferation data.

8. Both CA and MN were measured in nurses and patients, exposed to the cytotoxic drugs. Despite possible exposure levels there was little difference in the fold increase in cytogenetic endpoint, as CA and MN gave similar results (figure 2).

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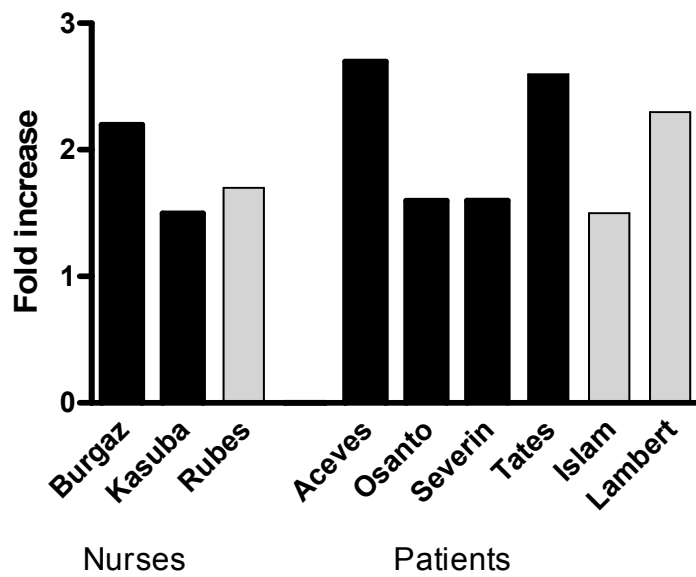


Figure 2. Comparison of magnitude of response for different cytogenetic endpoints, in nurses occupationally exposed to, and patients treated with cytotoxic agents. Cytogenetic analysis carried out by measuring MN (black bars) or CA (grey bars).

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Author	End point	Fold increase	Subject	Drug	Dose
Burgaz, et al., 1999	MN	2.2	Nurses	Cyclophosphamide	Urinary level = 0.02-9.14 □g/24 hr
Kasuba, et al., 1999	MN	1.5	Nurses	Cytotoxic drugs	
Rubes, et al., 1998	CA	1.7	Nurses and physicians	Cytotoxic drugs	
Aceves AFJ, et al., 2004	MN	2.7	Systemic lupus erythematosus patients	Cyclophosphamide	500 mg to 1 g/m ² total body surface
Islam, et al., 1993	CA	1.5	Ovarian cancer patients	Melphalan	
Lambert, et al., 1984	CA	2.3	Ovarian cancer patients	Melphalan	> 420 mg or < 420 mg. Data show a dose and time response
Osanto, et al., 1991	MN	1.6	Testicular carcinoma patients	Etoposide	
Severin, 1995	MN	1.6	Systemic lupus erythematosus patients	Cyclophosphamide	
Tates, et al., 1994	MN	2.6	Cancer patients	Cyclophosphamide	

Table 1. Fold increase in response following therapeutic exposure to IARC category 1 mutagens

Overall fold increase = 2.0

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Control data from biomonitoring studies in pesticide workers

9. Control data for MN (figure 3) and CA (figure 4) were collected from all biomonitoring studies with pesticide workers. The MN data were normally distributed (figure 5) whereas data for CA showed a skewed distribution (figure 6).

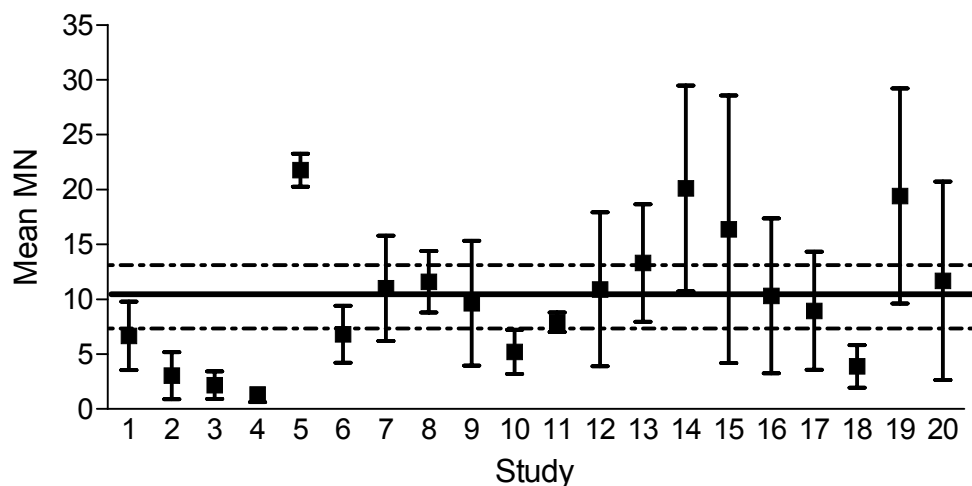


Figure 3. Control data for MN analysis. Graph shows mean (solid line) and 95 % CI (dashed lines)

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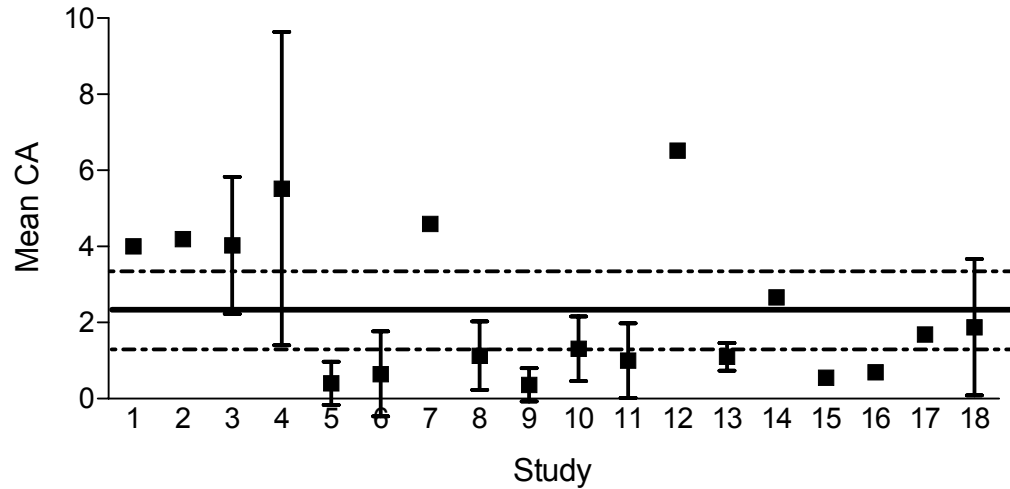
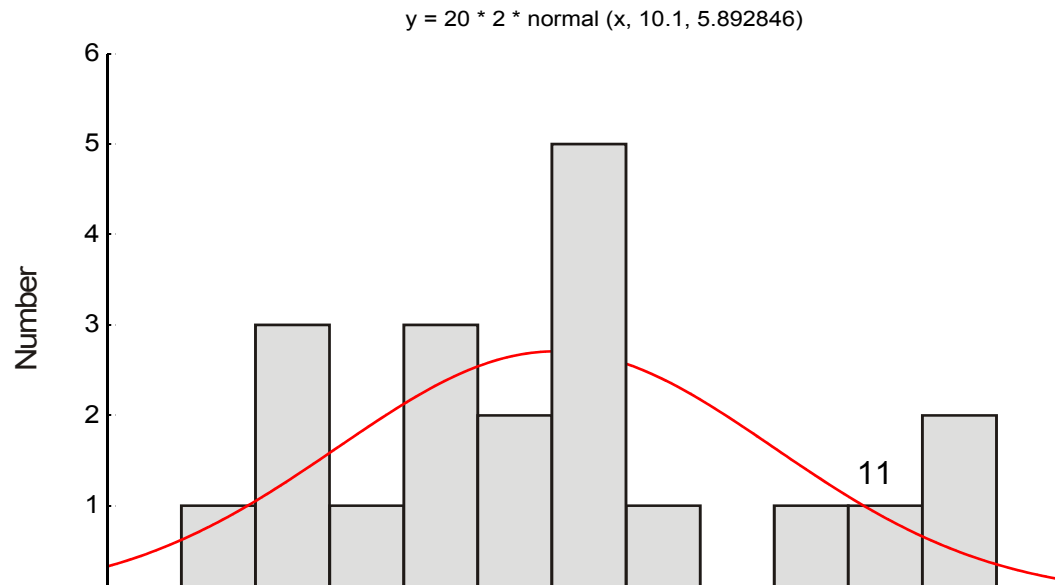
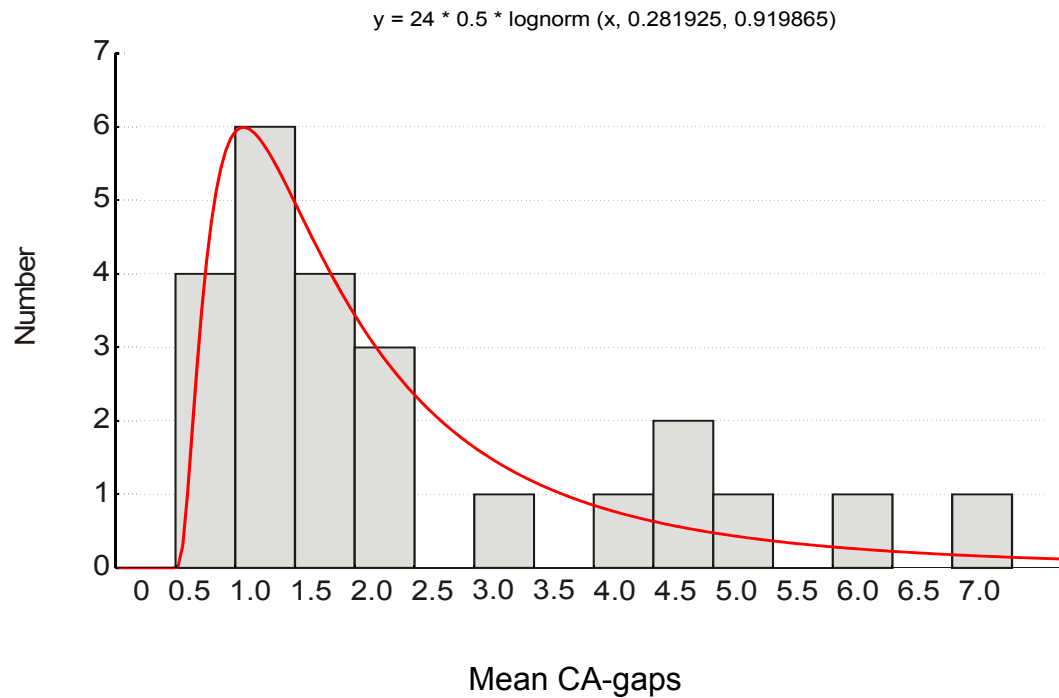


Figure 4. Control data for CA analysis. Graph shows mean (solid line) and 95 % CI (dashed lines)



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Figure 5. Distribution of MN data.



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Figure 6. Distribution of CA data.

10. Statistical parameters such as the pooled mean of all individual studies, the pooled standard deviation and 95 % CI were calculated (table 2).

11. The pooled mean was calculated by using equation $\frac{\sum(\bar{x}_n)}{\sum n}$

12. The pooled SD was calculated by using equation $\sqrt{\frac{\sum(SD^2 \times (n-1))}{\sum n-1}}$

Table 2. Control data for MN and CA assays

	MN	CA		
Mean ± SD	9.91 ± 6.15	2.73 ± 1.54		
SE	1.38	0.36		
Median	9.97	1.50		
95% CI	7.34 12.86	– 1.38 3.32	–	–
Range	1.32 21.76	– 0.36 6.52	–	–
25 % percentile	5.94	0.67		

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75 % percentile	12.50	4.11
Coefficient of variation	49 %	88 %
Fold increase between lowest and highest data	16.5	16.3

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References

Aceves AFJ, Esquivel Nava GA, Gallegos Arreola MP, Gomez Meda B, Zuniga Gonzalez G and C., R.-R. (2004). Cyclophosphamide boluses induce micronuclei expression in buccal mucosa cells of patients with systemic lupus erythematosus independent of cytochrome P450 2D6 status. *J Rheumatol* 31, 1335-9.

Albertini, R. J., Anderson, D., Douglas, G. R., Hagmar, L., Hemminki, K., Merlo, F., Natarajan, A. T., Norppa, H., Shuker, D. E., Tice, R., Waters, M. D. and Aitio, A. (2000). IPCS guidelines for the monitoring of genotoxic effects of carcinogens in humans. International Programme on Chemical Safety. *Mutat Res* 463, 111-72.

Burgaz, S., Karahalil, B., Bayrak, P., Taskin, L., Yavuzaslan, F., Bokesoy, I., Anzion, R. B., Bos, R. P. and Platin, N. (1999). Urinary cyclophosphamide excretion and micronuclei frequencies in peripheral lymphocytes and in exfoliated buccal epithelial cells of nurses handling antineoplastics. *Mutat Res* 439, 97-104.

Islam, M. Q., Kopf, I., Levan, A., Granberg, S., Friberg, L. G. and Levan, G. (1993). Cytogenetic findings in 111 ovarian cancer patients: therapy-related chromosome aberrations and heterochromatic variants. *Cancer Genet Cytogenet* 65, 35-46.

Kasuba, V., Rozgaj, R. and Garaj-Vrhovac, V. (1999). Analysis of sister chromatid exchange and micronuclei in peripheral blood lymphocytes of nurses handling cytostatic drugs. *J Appl Toxicol* 19, 401-4.

Lambert, B., Holmberg, K. and Einhorn, N. (1984). Persistence of chromosome rearrangements in peripheral lymphocytes from patients treated with melphalan for ovarian carcinoma. *Hum Genet* 67, 94-8.

Osanto, S., Thijssen, J. C., Woldering, V. M., van Rijn, J. L., Natarajan, A. T. and Tates, A. D. (1991). Increased frequency of chromosomal damage in peripheral blood lymphocytes up to nine years following curative chemotherapy of patients with testicular carcinoma. *Environ Mol Mutagen* 17, 71-8.

Rubes, J., Kucharova, S., Vozdova, M., Musilova, P. and Zudova, Z. (1998). Cytogenetic analysis of peripheral lymphocytes in medical personnel by means of FISH. *Mutat Res* 412, 293-8.

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Severin, E. (1995). Micronucleus frequency in peripheral blood lymphocytes from patients with systemic lupus erythematosus. Rom J Morphol Embryol 41, 55-8.

Tates, A. D., van Dam, F. J., Natarajan, A. T., Zwinderman, A. H. and Osanto, S. (1994). Frequencies of HPRT mutants and micronuclei in lymphocytes of cancer patients under chemotherapy: a prospective study. Mutat Res 307, 293-306.

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: Data reported in selected studies

Numbers in subscript refer to data on the figure 1.

13. Author	14. Results	15. Data for controls	16. Data for exposed subjects	17. Fold increase over controls
18. ^a Bolognesi <i>et al.</i> , 1993b	19. + MN	20. MN frequency = 6.67 ± 3.12 21.	22. MN frequency = 8.57 ± 5.02 ¹ 23. (mean / 1000 cells ± SD) 24. RR = 1.25; 95 % CI = 1.11 – 1.41	25. 1.3-fold increase
26. ^a Bolognesi <i>et al.</i> , 2004	27. - MN	28. Total MN (C+MN) = 2.18 ± 6.31 29. Total MN (C-MN) = 1.32 ± 3.38	31. Total MN (C+MN) = 2.79 ± 12.21 ² 32. Total MN (C-MN) = 1.56 ± 6.00 ³	34. 1.2-fold increase

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	30.	33. (mean / 1000 cells \pm SD)	35. 1.3-fold increase
			36.
37. ^a Carbonell <i>et al.</i> , 1995	38. + CA	49. Spring/summer	61.
	39. Spring/summer	50. Cells with aberrations = 4.56 \pm 2.53 % Chromatid-type aberrations = 3.14 \pm 2.76 %	62. 1.4-fold increase
	40. Cells with aberrations = 4.56 \pm 2.53 % Chromatid-type aberrations = 3.14 \pm 2.76 %	51. Chromatid-type aberrations = 5.31 \pm 3.12 %	63. 1.7-fold increase
	41. Chromosome-type aberrations = 1.90 \pm 1.51 %	52. Chromosome-type aberrations = 1.63 \pm 1.56 %	64.
	42. Total aberrations = 5.04 \pm 2.85 %	53. Total aberrations = 6.93 \pm 3.5 % ⁴	65. 0.9-fold increase
	43.	54.	66.
	44. Autumn/winter	55. Autumn/winter	67. 1.4-fold increase
	45. Cells with aberrations = 3.39 \pm 2.4 % chromatid-type aberrations = 2.57 \pm 2.0 %	56. Cells with aberrations = 3.69 \pm 2.14 %	68.
	46. Chromosome-type aberrations = 1.32 \pm 1.96 %	57. chromatid-type aberrations = 2.49 \pm 0.56 %	69.
	47. Total aberrations = 3.90 \pm 3.23 %	58. Chromosome-type aberrations = 1.21 \pm 1.83 %	70. 1.1-fold increase
	48.		71. 1.0-fold

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					59. Total aberrations = 3.70 ± 2.15 % 5	increase 72.
					60. (mean ± SD)	73. 0.9-fold increase 74.
						75. 1.0-fold increase
76. Ferrari et al., 1991	^a De et al.,	77. + CA 78. + SCE	79.	80. Chromatid-type aberrations = 4.44 ± 3.06 81. Chromosome-type aberrations = 1.08 ± 1.28 82. Complex rearrangements = <0.02 83. Total aberrations = 5.52 ± 4.12	84. Exposed subjects 85. Chromatid-type aberrations = 7.46 ± 6.22 86. Chromosome-type aberrations = 2.72 ± 1.58 87. Complex rearrangements = 0.12 ± 0.12 88. Total aberrations = 10.30 ± 7.18 ⁶ 89. 90. Exposed subjects with bladder cancer; 91. Chromatid-type aberrations = 5.07 ± 3.90	96. 97. 1.7-fold increase 98. 99. 2.5-fold increase 100. 101. 6.0-fold increase 102. 1.9-fold increase 103.

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92. Chromosome-type aberrations = 2.65 ± 0.26

93. Complex rearrangements = 0.30 ± 0.62

94. Total aberrations = 8.02 ± 4.98⁷

95. (Mean / 100 metaphases ± SD)

104.

105. 1.1-fold increase

106.

107. 2.5-fold increase

108.

109. 15.0-fold increase

110. **1.5-fold increase**

111. ^b Dulout <i>et al.</i> , 1985	112. + CA	113. Abnormal cells = 2.65 ± 1.01 %	114. Gaps = 1.56 ± 2.09	115. Chromatid breaks = 1.70 ± 0.74	116. Chromosome breaks = 0.54 ± 0.62	117. Dicentric chromosome and ring chromosome = 0.10 ± 0.21	118.	119. Abnormal cells = 2.71 ± 0.36 %	120. Gaps = 2.43 ± 1.62	121. Chromatid breaks = 1.51 ± 1.26 ⁸	122. Chromosome breaks = 0.95 ± 1.08 ⁹	123. Dicentric chromosome and ring chromosome = 0.43 ± 0.84 ¹⁰	124. (CA / 100 cells - gaps ± SD)	125. 1.0-fold increase	126. 1.6-fold increase	127. 0.9-fold increase	128. 1.8-fold increase	129.
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						130. 4.3-fold increase
						131.
132. ^a Falck <i>et al.</i> , 1999	133. + MN	134. MN frequency		137.		141.
		135. 0.5 μ g/ml BrdU = 7.4 \pm 3.1		138. 0.5 μ g/ml BrdU = 7.8 \pm 2.4 ¹¹		142. 1.1-fold increase
		136. 1 μ g/ml BrdU = 7.4 \pm 3.1		139. 1 μ g/ml BrdU = 8.0 \pm 2.7 ¹²		143. 1.1-fold increase
				140. (mean / 1000 cells \pm SD)		143. 1.1-fold increase
144. ^b Garry <i>et al.</i> , 1996	145. + CA	146. Rearrangement frequency = 0.4 \pm 0.57		147. Rearrangement frequency		152.
				148. Fumigant = 1.4 \pm 1.44 ¹³		153. 3.5-fold increase
				149. Insecticide = 1.4 \pm 1.28 ¹⁴		154. 3.5-fold increase
				150. Herbicide = 1.0 \pm 1.34 ¹⁵		154. 3.5-fold increase
				151. (mean \pm SD)		155. 2.5-fold increase
156. ^b Garry <i>et al.</i> , 2001	157. + CA	158. Translocations/inversions/deletions = 0.65 \pm 1.12		159. Translocations/inversions/deletions		163.
				160. Low volume (1-100 gall) = 1.20 \pm 1.13 ¹⁶		164. 1.9-fold increase
				161. Mid-range (100-1000 gall)= 1.00 \pm		165. 1.5-fold

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				1.13 ¹⁷		increase
				162. ₁₈ Heavy (>1000 gall) = 2.22 ± 1.14	166.	3.4-fold increase
167. ^b Gomez-Arroyo <i>et al.</i> , 2000	168. + MN	169. MN frequency = 0.38 ± 0.021	170. MN frequency = 1.01 ± 0.03 ¹⁹	171. (mean / 100 cells ± SD)		172. 2.7-fold increase
173. ^b Grover, <i>et al.</i> , 2003	174. + comet	175. Smokers	179. Smokers	180. Comet tail length = 18.26 ± 9.76 ²⁰	183.	184. 2.6-fold increase
		176. Comet tail length = 7.03 ± 11.46	181. Non-smokers	182. ₂₁ Comet tail length = 19.75 ± 14.48	185.	186. 1.9-fold increase
		177. Non-smokers				
		178. Comet tail length = 10.34 ± 13.25				
187. ^b Hogstedt <i>et al.</i> , 1980	188. - CA	189. Cell with aberrations = 4.6 %	190. Cell with aberrations = 4.2 % ²²			191. 0.9 -fold increase
192. ^a Kourakis <i>et al.</i> , 1992	193. + CA	194. Chromosome-type aberrations = 0.2 ± 0.37	197. Chromosome-type aberrations = 1.34 ± 1.62		201.	4.6-fold increase
		195. chromatid-type aberrations = 0.34 ± 0.60	198. chromatid-type aberrations = 0.80 ± 0.81		202.	
		196. Total aberrations = 0.54 ± 0.90 %	199. Total aberrations = 2.14 ± 1.62 %		203.	6.1-fold increase

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23

			200. (mean / 100 metaphases \pm SD)	204.
				205. 5.0-fold increase
^{206.} ^a Lander <i>et al.</i> , 2000	207. + CA	208. Pre-season	212. Pre-season;	222.
		209. Chromatid-type aberrations-gaps = 1.03 \pm 0.82	213. Chromatid-type aberrations-gaps = 0.87 \pm 0.95	223. 0.84-fold increase
		210. Chromosome-type aberrations-gaps = 0.28 \pm 0.45	214. Chromosome-type aberrations-gaps = 0.45 \pm 0.74	224.
		211. Total aberrations-gaps = 1.31 \pm 0.85 %	215. Total aberrations-gaps = 1.32 \pm 1.23 % ²⁴	225. 1.6-fold increase
			216.	226.
			217. Post-season	227. 1.0-fold increase
			218. Chromatid-type aberrations-gaps = 1.04 \pm 0.99	228.
			219. Chromosome-type aberrations-gaps = 0.34 \pm 0.56	229.
			220. Total aberrations-gaps = 1.37 \pm 1.20 % ²⁵	230. 1.2 (1.0) - fold increase
			221. (Mean / 100 metaphases \pm SD)	231.
				232. 0.8 (1.2) - fold increase

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						233.
						234. 1.0 (1.0) - fold increase
						235. compared with pre-season (compared with controls)
236. ^a Lebailly <i>et al.</i> , 1998	237. + comet	238. Beginning of spraying season = 30	240. Middle of spraying season = 43 ²⁶			242. 1.4-fold increase
		239. Beginning of spraying season = 30	241. End of spraying season = 36 ²⁷			243. 1.2-fold increase
244. ^a Lebailly <i>et al.</i> , 1998b	245. + comet	246. DNA damage	259. DNA damage			274.
		247. Before spraying	260. After spraying			275.
		248. Mixture of pesticides = 48 %	261. Mixture of pesticides = 56 % ²⁸			276. 1.2-fold increase
		249. Herbicides on wheat = 30 %	262. Herbicides on wheat = 28 % ²⁹			277. 0.9-fold increase
		250. Fungicides on wheat = 43 %	263. Fungicides on wheat = 35 % ³⁰			278. 0.8-fold increase
		251. Fungicides & insecticides on peas = 36%	264. Fungicides & insecticides on peas =			279. 1.1-fold increase
		252.	265. 39% ³¹			

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253.	Tail moment	266.		280.
254.	Before spraying	267.	Tail moment	281.
255.	Mixture of pesticides = 3.21	268.	After spraying	282.
256.	Herbicides on wheat = 2.30	269.	Mixture of pesticides = 3.92 ²⁸	283.
257.	Fungicides on wheat = 3.64	270.	Herbicides on wheat = 1.93 ²⁹	284. 1.2-fold increase
258.	Fungicides & insecticides on peas = 2.39	271.	Fungicides on wheat = 3.58 ³⁰	285. 0.8-fold increase
		272.	Fungicides & insecticides on peas = 3.16 ³¹	286. 0.9-fold increase
		273.	(mean)	287. 1.3-fold increase
288.	^a Lebailly <i>et al.</i> , 2003	289.	- comet	290.
				291.
				292.
				293.
				294.
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			4.35 ± 1.11 (2.16-5.85)		301. Evening after pesticide use = not measured	308.
					302. Following morning = 4.80 ± 2.57 (3.18-12.76) ³³	309.
					303. (Mean ± SD)	310. 1.1-fold increase (compared to before pesticide use)
311. ^a Munnia <i>et al.</i> , 1999	312. + DNA adducts	313. DNA adducts = 2.17 x 10 ⁹ ± 5.75 RAL		314. DNA adducts = 8.50 x 10 ⁹ ± 14.95 RAL ³⁴		316. 3.9-fold increase
				315. (Mean ± SD)		
317. ^a Mustonen <i>et al.</i> , 1986	318. - CA	319. Aberrant metaphases-gaps		322. Aberrant metaphases-gaps		326.
		320. Non-smokers; 1.5 ± 0.73		323. Non-smokers; 1.2 ± 1.5 ³⁵		327. 0.8-fold increase
		321. Smokers; 1.9 ± 1.2		324. Smokers; 1.8 ± 1.26 ³⁶		328. 1.0-fold increase
				325. (Mean ± SD)		
329. ^b Paldy <i>et al.</i> , 1987	330. + CA	331. Chromosome aberrations = 1.1 ± 0.36		333. Years of exposure		339.
		332.		334. Chromosome aberrations		340.
				335. 0-5 years = 2.96 ± 0.36 ³⁷		341. 2.7-fold increase
				336. 6-10 years = 3.55 ± 0.75 ³⁸		342. 3.2-fold

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				337. 11-15 years = 4.28 ± 0.76 ³⁹	increase
				338. (Sum of aberrations / 100 cells without gaps \pm SD)	343. 3.9-fold increase
344. ^a Pasquini <i>et al.</i> , 1996	345. + MN	346. MN frequency = 13.30 ± 5.35	347.	350. MN frequency (>19 year) = 18.30 ± 7.22 ⁴⁰	353. 1.37-fold increase
		348. Overall MN frequency = 13.30 ± 5.35	349.	351. Overall MN frequency = 15.98 ± 7.65 ⁴¹	354.
				352. (Mean / 1000 cells \pm SD)	355. 1.2-fold increase
356. ^a Pastor <i>et al.</i> , 2001b	357. - MN	358. MN frequency = 16.38 ± 12.19		359. MN frequency = 12.20 ± 6.58 ⁴²	360. 0.7-fold increase
361. ^a Pastor <i>et al.</i> , 2002a	362. - MN	363. MN frequency = 10.3 ± 7.06		364. MN frequency = 10.22 ± 7.06 ⁴³	365. 1.0-fold increase
366. ^a Peluso <i>et al.</i> , 1996	367. + DNA adducts	368. DNA adducts = 9		369. DNA adducts = 42 ⁴⁴	370. 4.7-fold increase
371. ^a Piperakis <i>et al.</i> , 2003	372. - comet	373. DNA damage		376. DNA damage	380.
		374. Male non-smokers = 82.3 ± 14.1		377. Male non-smokers = 83.2 ± 14.02 ⁴⁵	381. 1.0-fold increase
		375. Female non-smokers = $81.1 \pm$			

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16.12

378. Female non-smokers = 82.1 ± 382. 1.0-fold increase
13.14⁴⁶

379. (mean ± SD)

^a Studies from EU ^b Studies from rest of world

Mean fold increase of positive studies over controls ± SD (SE) = 1.73 ± 1.07 (0.15)

Figures in bold denote the total or mean fold increase of the study.

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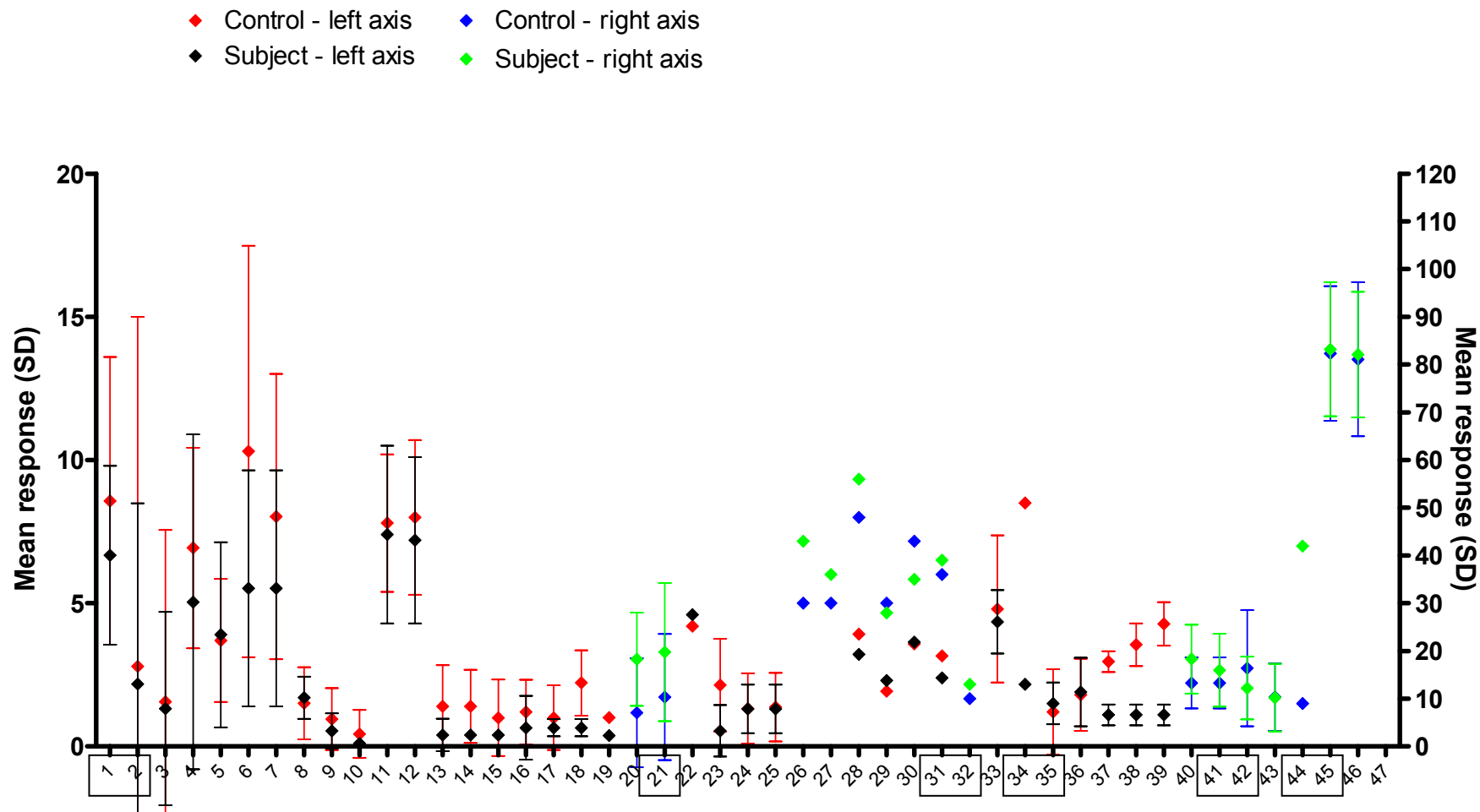


Figure 1. Graphical presentation of data from all selected studies. Data on CA, MN, DNA adducts and comet assay included.

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383. For details about each study, refer to the table. Data are presented as mean \pm SD. ^a mean $\times 10^9$ ^b mean $\times 10^2$

384. Studies in boxes presented negative data.

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385. Some data sets on the graph represent data that appear to be significantly different.

386. Garry *et al.*, 2001 (18): Workers carrying out heavy pesticide use (> 1000 gallons)

387. Paldy *et al.*, 1987 (37-39): Workers exposed to pesticides for 0-5, 6-10 or 11-15 years.

388.

389.

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ANNEX 2 TO MUT/05/12

Pesticide usage: comparison between pesticide use in selected studies of pesticide applicators and practices in UK.

1. Twenty-four studies have been selected based on various exclusion criteria. An attempt was made to identify active ingredients that may contribute to the statistically significant cytogenetic effects observed in some of the studies. It is recognised that although statistical significance was demonstrated, biological significance of data has yet to be ascertained.
2. From the 24 selected studies, data on the active ingredients used in the study was reported in 14 studies. Of these, 11 studies reported positive data and 3 reported negative data.
3. Two approaches were used to try to correlate pesticides used in the selected studies with those used in agricultural and floricultural practice in the UK. The information retrieved is intended to aid the COM in its discussions of possible chemical exposures associated with the positive results reported in some of the selected studies. No full review of the mutagenicity of pesticide active ingredients has been undertaken as this is outside the scope of the current referral from the ACP. Preliminary information has been obtained from consultation with HSE on the classification status under Directive EC/67/548.

Identification of pesticides used in positive studies: comparison with Pesticide Usage Survey

4. A quantitative comparison of the pesticides groups reportedly used in the positive and negative studies was carried out, although it is recognised that this is a crude measurement of pesticide exposure.
5. The reported use of pyrethroids, thiocarbamates and benzimidazoles was higher in positive studies compared to negative studies. At least one pyrethroid or benzimidazole was cited in 73 % of the positive studies, compared to 33 % of negative studies and at least one thiocarbamate was cited in 36 % of positive studies whereas none was used in negative studies.
6. Pyrethroids cited included bifenthrin, cypermethrin, deltamethrin, fenpropathrin, fenvalerate, permethrin and tralomethrin. Thiocarbamates reportedly used included maneb, thiram and metam-sodium, and benzimidazoles used included benomyl, carbendazim, thiabendazole and thiophanate methyl. [There are no currently approved pesticide products in the UK for use in agriculture/horticulture using fenvalerate, permethrin, benomyl, tralomethrin]
7. From the above list of active ingredients, bifenthrin, cypermethrin, deltamethrin, fenpropathrin, maneb, thiram, metam-sodium, carbendazim, thiabendazole and thiophanate methyl are approved for use in pesticide

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products in the UK, according to the information retrieved from the PSD internet site. Fenvalerate and tralomethrin could not be found on the PSD internet site. In the list of active substances covered by the EC Review Programme, updated in 10th October 2003 both fenvalerate and tralomethrin were listed as being active substances not supported or withdrawn in stages 1-3 of the EC Review Programme.

8. No approved products were found on the PSD database containing permethrin and benomyl. However, in the list of active substances covered by the EC Review Programme, updated in 10th October 2003, both were listed as being approved for use in UK. Furthermore, permethrin was listed as a amateur product withdrawn from the market during stage 1 of the EC Review Programme. The last day for use and storage of permethrin was 27th June 2002.

9. The Pesticide Usage Survey reports data for the top 50 most used active ingredients, according to area sprayed and amount used. In contrast to the selected studies, who report data for a specific occupational group i.e. floriculturist/greenhouse worker, farmer/agriculturist, the Pesticide Usage Survey gives data for different crops i.e. protected crops, outdoor vegetables crops. For the purpose of this report, it has been assumed that data for protected crops, outdoor bulbs and flowers, and hardy nursery stock relate to floriculturists and greenhouse workers and data for mushroom crops, outdoor vegetable crops, commercial grain, farm grain, arable crops, orchards and fruit stores and grassland-fodder relate to agriculturists and farmers.

Floriculturists and greenhouse workers

10. The active ingredients that are listed in the top 50 used in the UK for protected crops, outdoor bulbs and flowers and hardy nursery stock are shown in figure 1-3.

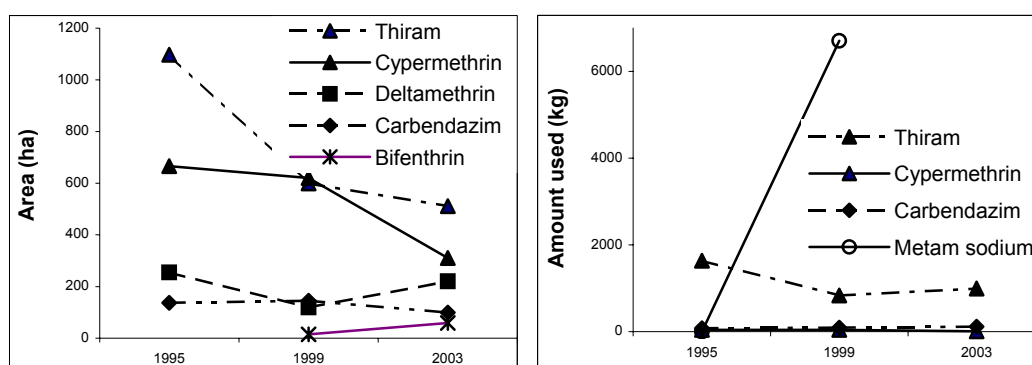


Figure 1. The area sprayed (left) and amount of pesticides used (right) on protected crops. *metam-sodium was not listed in the top 50 in 1995

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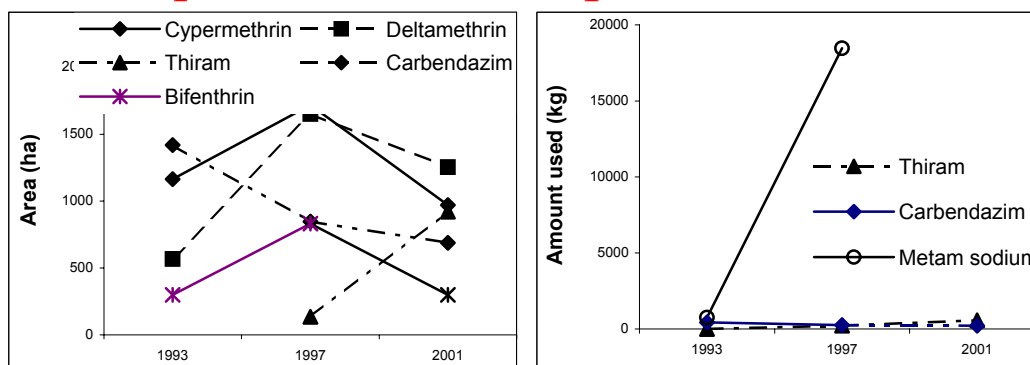


Figure 2. The area sprayed (left) and amount of pesticides used (right) on hardy nursery stock.

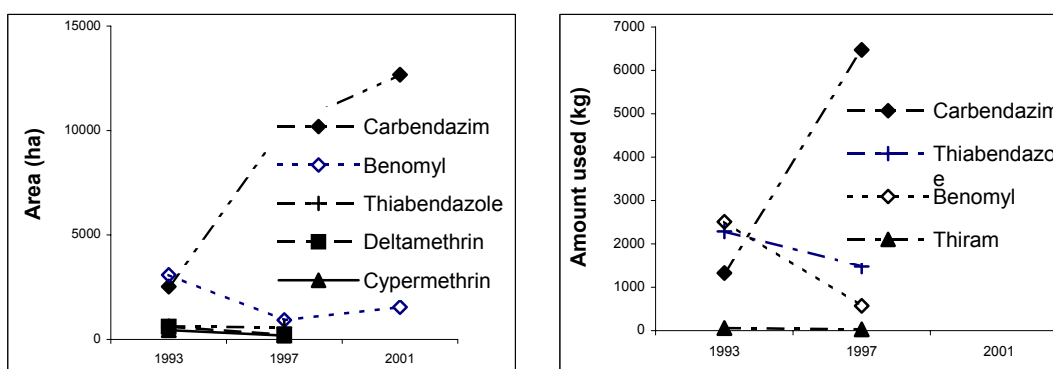


Figure 3. The area sprayed (left) and amount of pesticides used (right) on outdoor flowers and bulbs.

11. The amount of metam sodium used on protected crops and hardy nursery stock increased between 1995-1999 and 1993-1997, respectively, although they were not listed in the top 50 most used pesticides in 2003 or 2001. In addition, the area of outdoor bulbs and flowers sprayed with carbendazim and the amount of carbendazim used also increased. All of the other pesticides listed in the top 50 either decreased during the time period studied or the amounts used remained the same.

Agriculturists and farmers

12. The active ingredients that are listed in the top 50 used in the UK for agricultural crops shown in figure 4-11.

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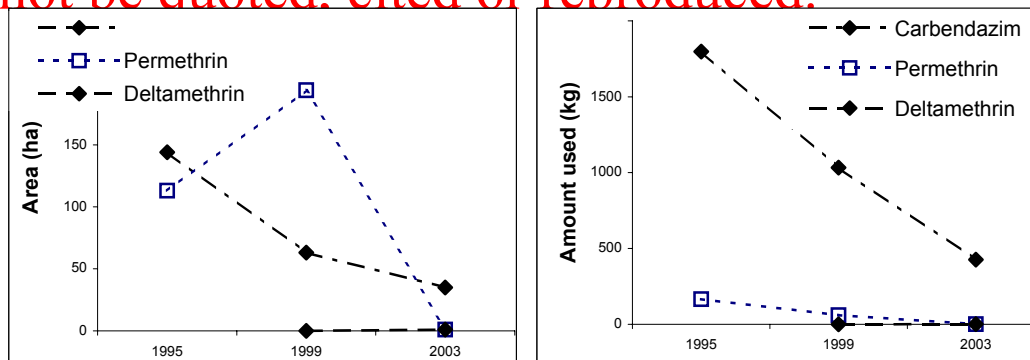


Figure 4. The area sprayed (left) and amount of pesticides used (right) on mushroom crops.

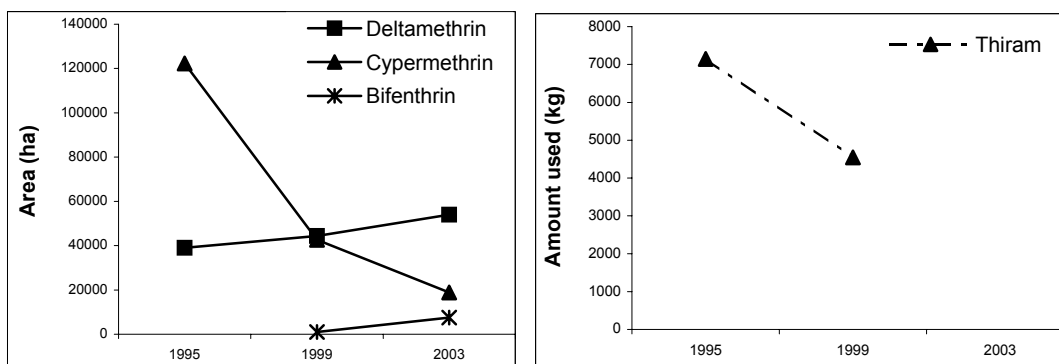


Figure 5. The area sprayed (left) and amount of pesticides used (right) on outdoor vegetable crops.

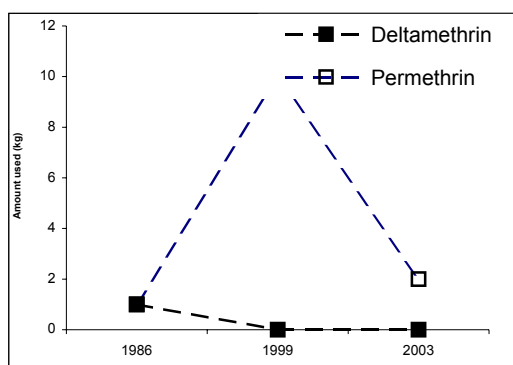


Figure 6. The amount of pesticides used on commercial grain.

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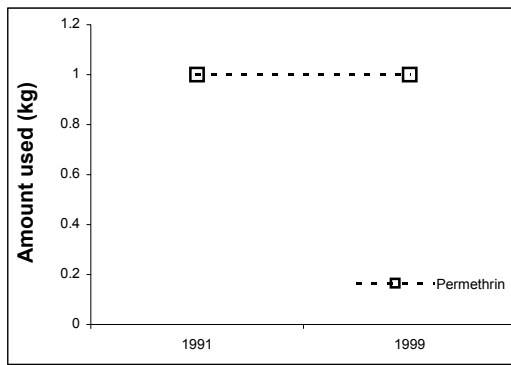


Figure 7. The amount of pesticides used on farm grain.

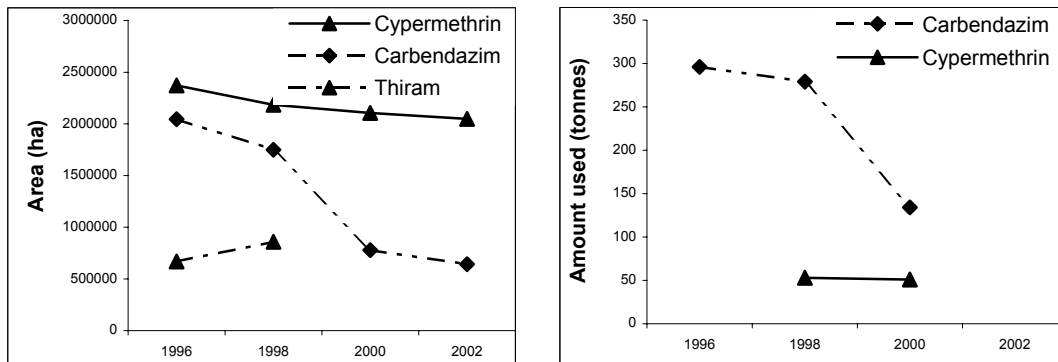


Figure 8. The area sprayed (left) and amount of pesticides used (right) on arable crops.

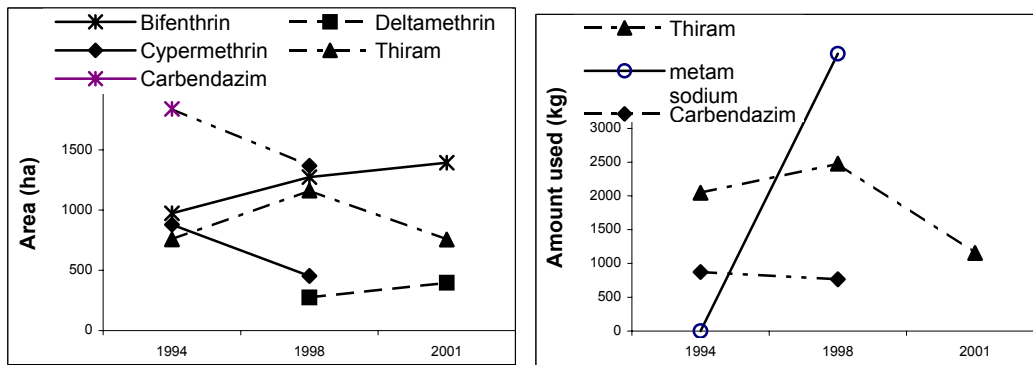


Figure 9. The area sprayed (left) and amount of pesticides used (right) on soft fruit. *metam-sodium was not listed in the top 50 in 1994.

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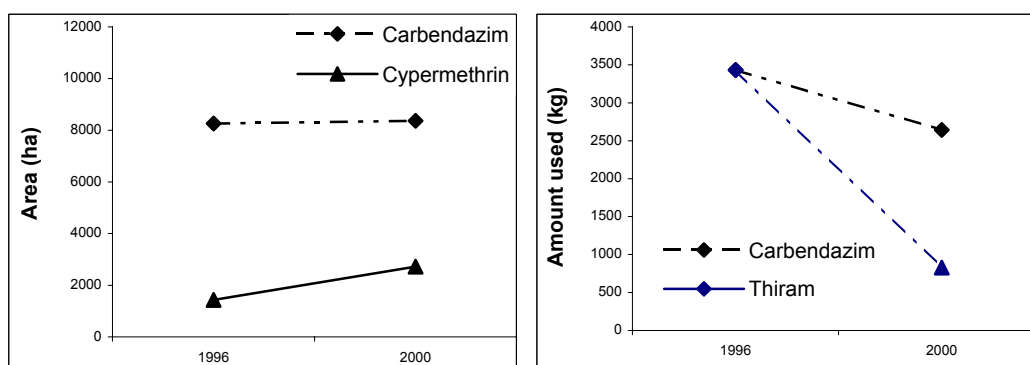


Figure 10. The area sprayed (left) and amount of pesticides used (right) on orchards and fruit stores.

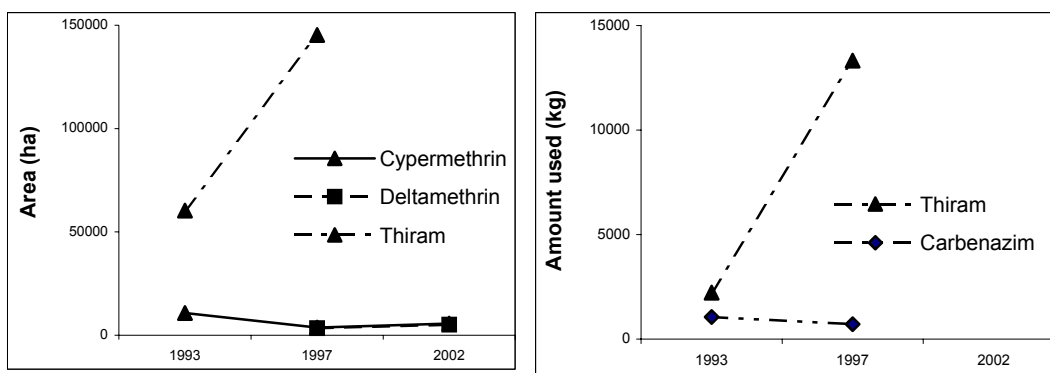


Figure 11. The area sprayed (left) and amount of pesticides used (right) on grassland/fodder.

13. The area of soft fruit sprayed with bifenthrin increased between 1994 and 2001 and the amount of metam sodium used on soft fruit increased between 1994-1998, although it was not listed in the top 50 most used pesticides in 2001. In addition, the area of grassland and fodder sprayed with thiram and the amount of thiram used increased during 1993 and 1997, although it was not listed in the top 50 active ingredients used in 2002. All of the other pesticides listed in the top 50 either decreased during the time period studied or the amounts used remained the same.

Pesticides identified as category 2 or 3 mutagens and cited in selected studies

14. A number of pesticide active ingredients are included in Annex I of the Dangerous Substances Directive EC/67/548 as category 2 or 3 mutagens (i.e. in-vivo mutagens (category 3) and in-vivo germ cell mutagens in animals

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(category 2)) This list includes, benomyl (category 2), carbendazim (category 2), cycloheximide, diuron, DNOC (4,6-dinitro-o-cresol), edifenphos, fenthion, methyl bromide, monocrotophos, phosphamidon, and thiophanate methyl,

15. Fenthion, monocrotophos, benomyl, carbendazim, thiophanate methyl, DNOC, and diuron were reportedly used in some of the selected studies of pesticide applicators. [There are no currently approved products for use in the U.K. in agriculture/horticulture using fenthion, monocrotophos, benomyl, DNOC)

16. Organophosphorus compounds fenthion and monocrotophos were used in 1/11 and 5/11 positive studies respectively, and no negative studies.

17. Benzimidazoles, carbendazim and thiophanate methyl were used in, 5/11 and 1/11 positive studies respectively.

18. Dinitrophenol compound DNOC was used in 1/11 positive study and no negative studies and diuron, an amide, was used in 1/11 positive study and one negative study.

19. From the 11 selected studies that reported positive data, 10 studies reported the use of at least one active ingredient classified as a category 2 or 3 mutagen (table 1).

20. Studies by Pasquini *et al.*, 1996 and Dulout *et al.*, 1985 reported the use of one listed pesticide. Carbonell *et al.*, 1995, De Ferrari *et al.*, 1992, Grover *et al.*, 2003, Gomez-Arroyo *et al.*, 2000, Lander *et al.*, 2000 and Lebailly *et al.*, 1998b all used two of the listed pesticides. Peluso *et al.*, 1996 reported the use of three listed pesticides and Bolognesi *et al.*, 2004, used four of the pesticides classified as category 2 or 3 mutagens. Workers in the remaining positive study by Garry *et al.*, 2001 were exposed to 2,4-D and possibly also to a mixture of other pesticide active ingredients.

21. From the three negative studies that reported pesticide exposure, only Lebailly *et al.*, 2003 reported the use of two category 3 mutagenic active ingredients, whereas workers in the studies carried out by Falck *et al.*, 1999 and Pastor *et al.*, 2001a did not use any of the listed pesticides.

Table 1. Studies that used active ingredients classified as category 3 mutagens.

Active ingredient	Chemical group	Positive studies that used active ingredient	Negative studies that
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			used active ingredient
Fenthion*	Organophosphorus	Carbonell, <i>et al.</i> , 1990	
Monocrotophos*	Organophosphorus	Bolognesi, <i>et al.</i> , 2004, De Ferrari, <i>et al.</i> , 1991, Dulout, <i>et al.</i> , 1985, Grover, <i>et al.</i> , 2003, Peluso, <i>et al.</i> , 1996	
Benomyl*	Benzimidazole	Bolognesi, <i>et al.</i> , 2004, Carbonell, <i>et al.</i> , 1995, Gomez-Arroyo, <i>et al.</i> , 2000, Lander, <i>et al.</i> , 2000, Pasquini, <i>et al.</i> , 1996, Peluso, <i>et al.</i> , 1996	Lebailly, <i>et al.</i> , 2003
Carbendazim	Benzimidazole	Bolognesi, <i>et al.</i> , 2004, Grover, <i>et al.</i> , 2003, Lander, <i>et al.</i> , 2000, Lebailly, <i>et al.</i> , 1998, Peluso, <i>et al.</i> , 1996	
Thiophanate methyl	Benzimidazole	Bolognesi, <i>et al.</i> , 2004	
DNOC*	Dinitrophenol	De Ferrari, <i>et al.</i> , 1991	
Diuron	Amide	Gomez-Arroyo, <i>et al.</i> , 2000	Lebailly, <i>et al.</i> , 2003

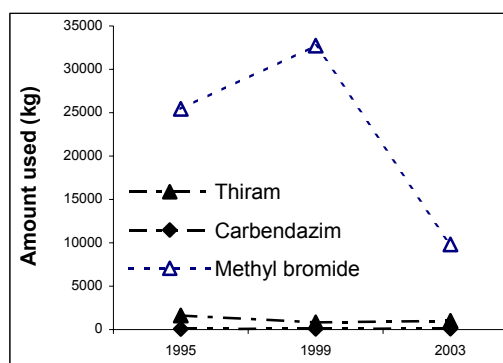
- Pesticide active ingredients with no products currently approved in the U.K.
- Products containing carbendazim, diuron, and thiophanate-methyl currently approved for use in the U.K.

Pesticides identified as category 2 or 3 mutagens: comparison with Pesticide Usage Survey

22. As mentioned above, a number of active ingredients have been classified as category 3 mutagens. Several of these pesticides have been listed as being in the top 50 pesticides used, in terms of area sprayed or amount (kg) used.

Floriculturists and greenhouse workers

23. The active ingredients, classified as category 3 mutagens, which are



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listed in the top 50 used in the UK for protected crops, outdoor bulbs and flowers and hardy nursery stock are shown in figure 12-14. [Note thiram is not listed as a category 3 mutagen]

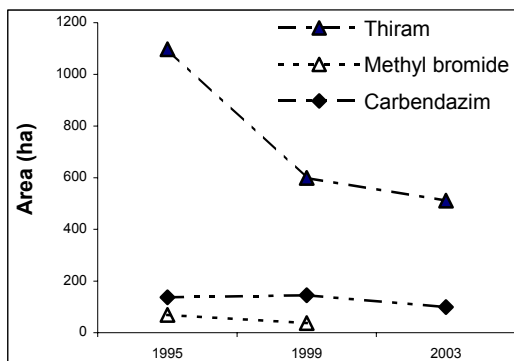


Figure 12. The area sprayed (right) and amount of pesticide sprayed (right) on protected crops.

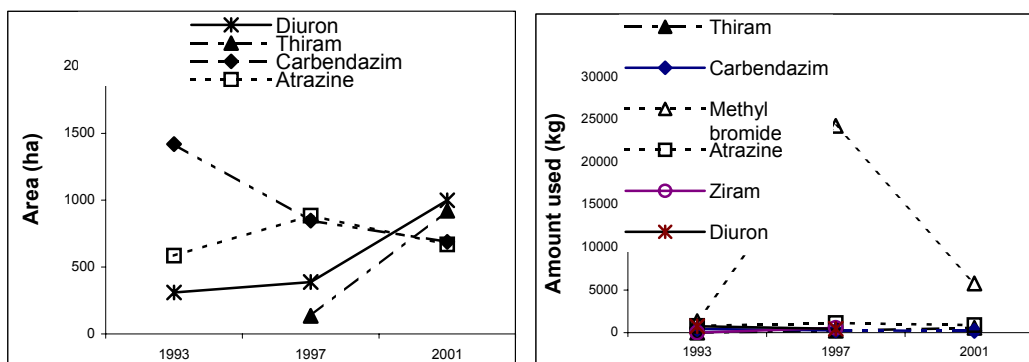


Figure 13. The area sprayed (left) and amount of pesticide used (right) on hardy nursery stock. *ziram was not listed in the top 50 in 1993.

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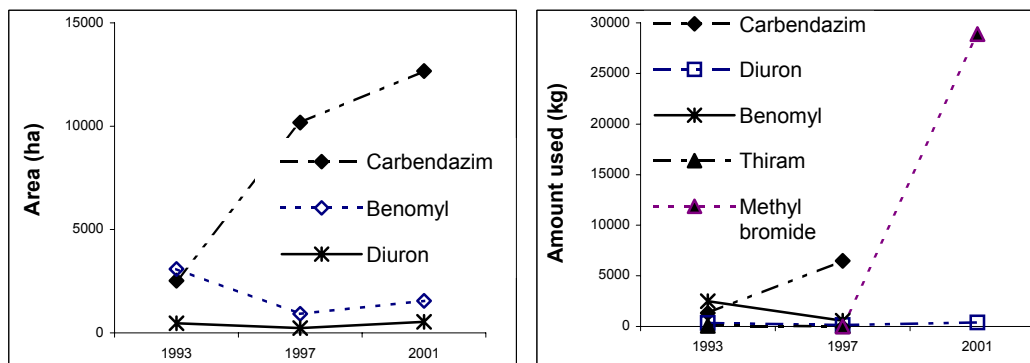


Figure 14. The area sprayed (left) and amount of pesticide used (right) on outdoor bulbs and flowers. *methyl bromide was not listed in the top 50 in 1995

24. The area of outdoor bulbs and flowers sprayed with carbendazim and the amount of methyl bromide (kg) used increased between 1993 and 2001. In addition, the area of hardy nursery stock sprayed with diuron increased between 1993 and 2001. All of the other pesticides listed in the top 50 either decreased during the time period studied or the amounts used remained the same.

Agriculturists and farmers

25. The active ingredients, classified as category 3 mutagens, which are listed in the top 50 used in the UK for agricultural crops shown in figure 15-19.

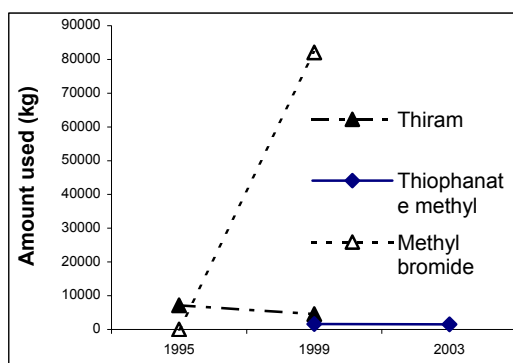


Figure 15. The amount of pesticides used on outdoor vegetable crops. *methyl bromide was not listed in the top 50 in 1995.

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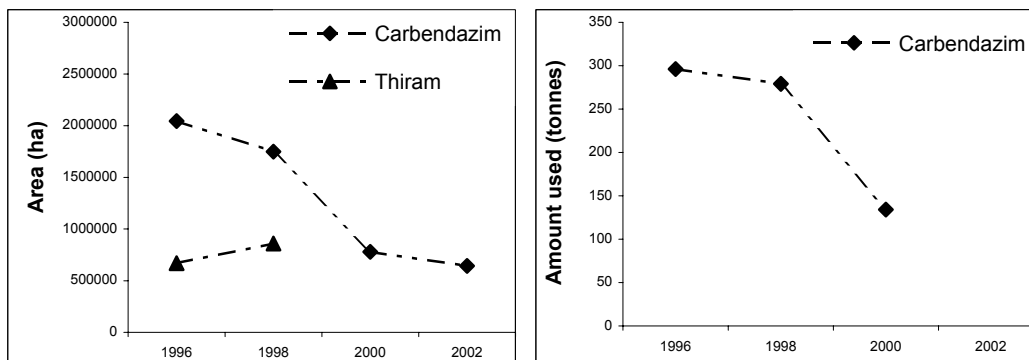


Figure 16. The area sprayed (left) and amount of pesticide used (right) on arable crops.

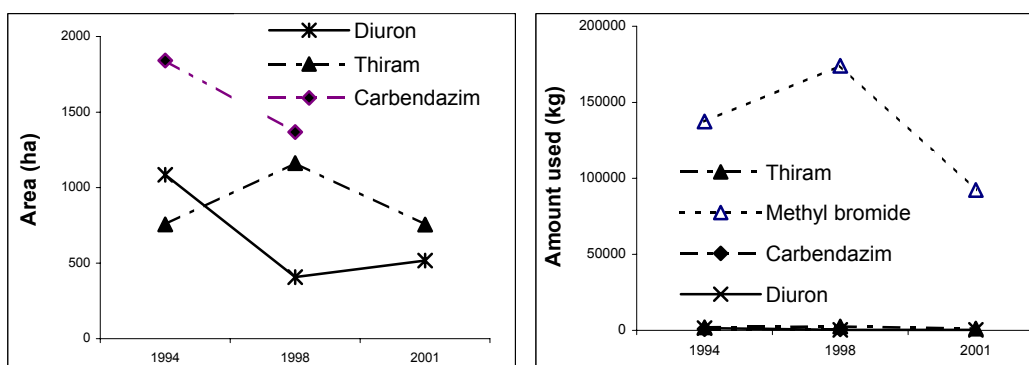


Figure 17. The area sprayed (left) and amount of pesticide used (right) on soft fruit.

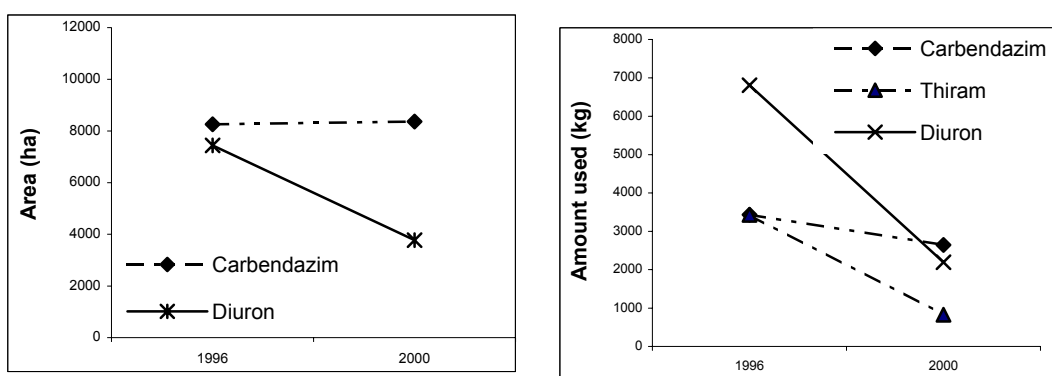


Figure 18. The area sprayed (left) and amount of pesticide used (right) on orchards and fruit stores.

This is a draft paper for discussion. It should not be quoted, cited or reproduced.

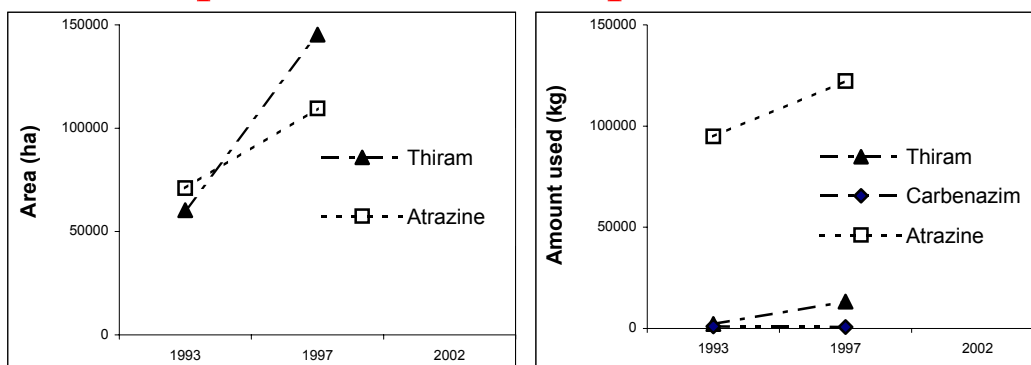


Figure 19. The area sprayed (left) and amount of pesticide used (right) on grassland and fodder.

26. The amount of methyl bromide used on outdoor vegetable crops increased between 1995-1999, although it was not listed in the top 50 most used pesticides in 2003. All of the other pesticides listed in the top 50 either decreased during the time period studied or the amounts used remained the same.

Discussion/Conclusion

27. To conclude, several active ingredients that were identified as being used by workers in the positive selected studies that originate from EU countries and from other countries in rest of the world, but from reported information appear to be used in some UK practices. The use of carbendazim in floricultural practices has increased over the past decade. In addition, in agricultural practices, the area sprayed with bifenthrin increased between 1994 and 2001.

28. Several active ingredients were identified as category 2 or 3 mutagens. The use of carbendazim, methyl bromide and diuron by floriculturists and there is evidence that the area sprayed with these pesticides in the U.K. has increased over the past decade. However, the use of none of these active ingredients increased in agricultural practices.

29. Ten out of the 11 positive selected studies that presented data regarding active ingredients used, reported the use of at least one active ingredient that is classified as a category 2 or 3 mutagen, whereas one of the three negative studies used such pesticides.

30. The COM are asked to consider a number of questions detailed in the covering paper MUT/05/12.