Committee on
MUTAGENICITY

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

STATEMENT: REVIEW OF BIOMONITORING STUDIES OF GENOTOXICITY IN PESTICIDE APPLICATORS.

COM/05/S5 – October 2005

Background

1. The Medical and Toxicology panel of the Advisory Committee on Pesticides has asked the COM for advice on the genotoxicity in pesticide applicators. The referral statement is given below.

"*To review investigations of mutagenicity and DNA adducts in pesticide applicators and workers exposed to pesticides (e.g. handling cut flowers) and factory (manufacturing) staff engaged in pesticide manufacture/formulation and produce a statement for the Advisory Committee on Pesticides and its Medical and Toxicology Panel (MTP) . The review should include all studies identified by the MTP and any other relevant studies published, particularly those originating from the UK.*

The review should consult COC epidemiologists with regard to the rigour of studies evaluated. this should include design, selection of controls, bias, confounding and use of multiple statistical comparisons.

The review should be initiated at the October 2004 COM meeting."

2. The referral from ACP and the MTP came about through the ongoing routine review of epidemiology literature undertaken by the MTP. The MTP had considered in April 2004 that there were sufficient numbers of reports of biomonitoring studies in the published literature which had been retrieved and evaluated by MTP to request an independent view from COM on the available studies. It is noted that pesticide active ingredients that are DNA reactive *in-vivo* mutagens are not approved for use in formulated pesticide products in the U.K.

Evidence reviewed

3. The DH Toxicology Unit at Imperial college in collaboration with the COM secretariat drafted a series of review papers for the COM. The review considered published biomonitoring studies in the scientific literature up to December 2004. A comparison was undertaken with the literature search strategy used by the MTP in order to ascertain whether any published studies had been missed. A very good agreement between the MTP and COM literature searches was attained with only one study identified in the COM

literature search which had not already been identified by MTP. (A total of 70 biomonitoring studies of genotoxicity markers in pesticide applicators was identified.[1-70]) A listing of the discussion papers considered by COM during the review period (from the October 2004 to the October 2005 meetings) is given below. All of these review papers are draft discussion papers and do not necessarily represent the views of the COM. A detailed evaluation was undertaken for all of the studies. The discussion papers can be accessed via the COM internet site under the "papers" section. (http://www.advisorybodies.doh.gov.uk/com/) ;

i) Biomonitoring studies from EU (MUT/04/19). Annex 1 (overview of literature), Annex 2 Summary of individual studies and IPCS guidelines on biomonitoring studies of genotoxicity. Annex 3 tabular summary according to occupation, Annex 4 tabular summary of statistical approaches used to analysis of data.

ii) Review of biomonitoring studies of pesticide applicators from Croatia (MUT/04/20)

iii) Further information and follow-up of review undertaken in October 2004 (MUT/05/1). (Draft exclusion criteria Annexes I and II, Draft inclusion criteria Annexes III and IV, Magnitude of response Annexes V and VI, Exposure patterns documented in studies).

iv) Submitted published papers (for February 2005 meeting) (MUT/05/6), Addendum 1 MUT/05/6 review of studies from rest of world, Addendum 2 to MUT/05/6, tabulation of rest of world studies by occupation.

v) Cytogenetic changes following cumulative exposure to pesticides (MUT/05/9)

vi) Revised criteria (MUT/05/10)

vii) Epidemiological overview (MUT/05/11)

viii) Discussion paper on evaluation of positive studies and control data (MUT/05/12) (Annex 1 Evaluation of positive response in biomonitoring studies of genotoxicity, Annex 2 Information on pesticide usage in UK).

Pesticide applications considered in the review.

4. The papers retrieved identified a wide diversity of occupational pesticide exposures. The authors described investigations in occupational groups such as floriculturalists, green house workers, agricultural workers and farmers, pesticide sprayers and applicators (which included agricultural/horticultural, amenity, fumigators), production workers (e.g. manufacture of pesticides) and forestry workers. The extent of information provided on occupational exposure to pesticides (e.g. during handling, diluting, applying), the duration of exposure and use and adequacy of personal protective clothing varies considerably between the different accounts. The Committee considered it was difficult to evaluate such a diverse data set. It was agreed that the most appropriate approach would be to assess the adequacy of the studies with regard to investigation and evaluation of genotoxicity indices and with regard to overall adequacy of design, analysis and interpretation of results. With respect to overall adequacy, the COM sought an opinion from an independent epidemiologist.

Indices of genotoxicity used in the reviewed biomonitoring studies

5. A short overview of the indices of genotoxicity used in biomonitoring studies is provided to assist in evaluating the significance of findings. Almost all the studies considered in this review provided data for investigations using *in-vitro* culture of peripheral blood lymphocytes derived from blood samples. A small number of studies used epithelial cells from the buccal cavity. The committee had access to the general guidance published by a WHO IPCS working group on use of genotoxicity indicators in biomonitoring studies.[71] The Committee agreed that in general the genotoxicity indices measured in samples (predominantly peripheral blood lymphocytes) including micronucleus formation, chromosomal aberrations, comet and, ^{32}P-postlabelled DNA adducts results indicate uptake and exposure to DNA damaging chemicals. The evidence suggested that there may be an increased risk of mutagenicity and also possibly carcinogenicity but it is not possible to be certain that there is a risk or to quantify this risk because of the poor quality of many of the studies and frequent contradictory findings.

Micronucleus frequency

Micronuclei are small, extranuclear bodies that arise from acentric chromosome fragments or from whole chromosomes that are excluded from the nucleus during mitotic cellular division. They can be a consequence of DNA breakage, replication on a damaged DNA template or inhibition of DNA synthesis, failure of any of the mitotic apparatus or alterations in cellular physiology and mechanical disruption [71,]. In most cases, the cytokinesis-block MN method is used, in which scoring only takes place in cells that have only divided once in culture.[16] Micronucleus analysis can be used for a number of cells, both *in vitro* and *in vivo*, including lymphocytes[5,9,72] and buccal epithelial cells.[49-50] Micronucleus induction is an indirect indicator of mutagenicity. It is unclear however, whether MN formation has a specific role in carcinogenesis.[71]

Chromosome aberrations

Structural chromosome aberrations arise from direct DNA breakage, replication on a damaged DNA template or inhibition of DNA synthesis and may involve both chromatids of the chromosome (chromosome-type CA), or only one chromatid of the chromosome (chromatid-type CA).[71,73] Chromosome aberration analysis has been commonly performed on human peripheral blood lymphocytes to assess DNA damage.[11,14,16,29,42,44] To ensure that only first-generation metaphase cells are scored for CA, bromodeoxyuridine is commonly added to the culture medium prior to DNA replication *in vitro* [29]*.* Both structural and numerical chromosome aberrations may cause alterations to the structure or arrangement of oncogene and tumour suppressor genes of somatic cells, and hence are involved in the induction of cancer in humans.[74]

Sister chromatid exchange

Sister chromatid exchanges arise from equal exchange of DNA replication products between two identical sister chromatids of a duplicated chromosome[75] They are thought to arise as a consequence of "error free" homologous recombinational repair or bypass of DNA lesions during replication on a damaged DNA template, possibly at the replication fork.[75] In the most commonly used method of SCE analysis, DNA replication is required for two consecutive cell cycles, hence bromodeoxyuridine is added to the culture medium and cells are scored in the second division metaphase.[14,61] Although the induction of SCE has been widely used as an indicator of DNA damage following exposure to pesticides,[12,26,32,36,45,61] the mechanism of

formation and biological significance of SCEs are still unknown.[75]. The COM agreed that biomonitoring studies using SCE analysis were not informative with regard to evidence for genotoxicity.

Comet assay

The comet assay, or single cell gel electrophoresis technique is a more recent technique established as a sensitive method for detecting DNA single strand and double strand breaks, alkali-labile sites, DNA cross linking and incomplete excision repair events.[34,64,76,77] The comet assay can be carried out with a number of cells, both *in vitro* and *in vivo*, including peripheral blood leukocytes, bladder, liver, buccal, gastric and sperm cells. To date, peripheral blood lymphocytes are mainly used for human biomonitoring studies following occupational exposure to an array of chemicals.[34,53,64,77]. However, the relevance of the endpoint measured in the comet assay has yet to be established, as it is usually the result of a temporary strand breakage, which is repaired within a few hours under normal circumstances and may or may not become fixed as a mutation.[77]

DNA adducts

A DNA adduct is a chemical entity covalently bound to DNA[71], and is usually formed following the interaction of an electrophilic molecule with a nucleophilic site of DNA[78]. They are often the initial DNA lesion following exposure to a genotoxic chemical and may lead to mutation and altered gene function if not repaired. In epidemiological studies DNA adducts are particularly useful as they provide information on the exact chemical exposure of the individual.[78] ^{32}P-DNA postlabelling technique has been widely used to measure non-radioactive carcinogenic large DNA adducts in humans, due to it being a highly sensitive technique.[78]

The COM guidance on a strategy for testing chemicals for mutagenicity recognised that artifactual positives may be obtained in the cell assays that do not reflect intrinsic mutagenic activity. Factors such as hyperthermia, hypothermia or induction of erythropoiesis may produce MN or CA[79] or exercise immediately prior to sampling may lead to increased DNA damage measured by the comet assay.[80,81]

Overview of approach used by COM

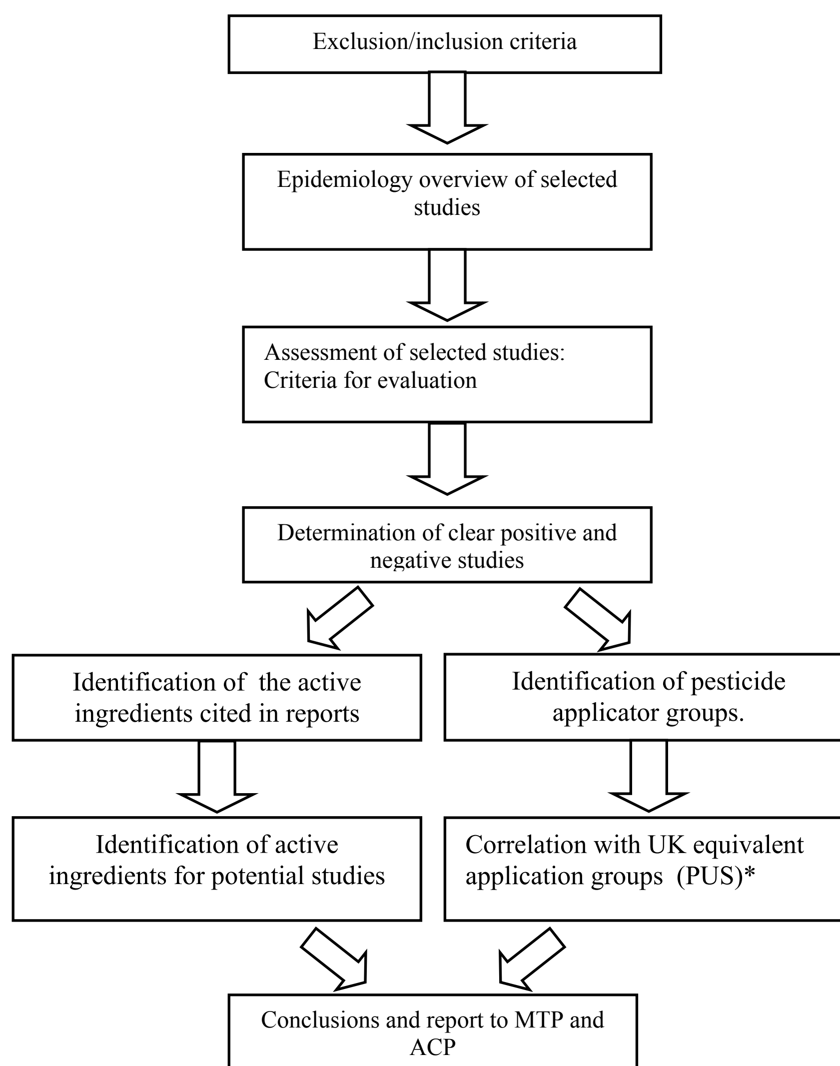
6. A flow diagram outlining the approach used by COM is shown below in Figure 1.

7. The COM took its remit from the referral from MTP and agreed to consider data originating from applications of formulated pesticides and pesticide mixtures. COM did not consider studies where a single pesticide active ingredient with known mutagenic potential was used (e.g methyl bromide fumigation). The COM agreed to eliminate a number of publications arising from studies undertaken in Croatia because of irregularities in the reported data. A number of papers from Zeljezic and Garaj-Vrhovac from the laboratory of Mutagenesis, Institute for Medical Research and Occupational Health, Zagreb, Croatia were published during 2000-2 and were identified during literature searches.[65-70] It was noted that there were apparent discrepancies between the different publications in the reporting of the demographic data on exposed and controls and reporting of results of mutagenicity studies and no confidence could be attached to the results.

8. The remaining 65 studies were undertaken from all parts of the world but the literature search did not find any published study which had evaluated UK pesticide applicators. The 65 studies were subject to a review procedure

for the adequacy of the genotoxicity assessment. The COM discussed the exclusion/inclusion criteria at the October 2004 and February 2005 meeting and following a postal consultation after the February 2005 meeting. The criteria and selected studies are outlined in Annex 1 to this statement.

Figure 1: Flow Diagram of approach used by COM.



* = Pesticide Usage Survey data

Epidemiology overview of biomonitoring studies

9. The 24 studies selected by COM using the exclusion/inclusion criteria were subjected to an epidemiology overview (see para 3 vii above). The full report can be accessed from the COM internet site (http://www.advisorybodies.doh.gov.uk/pdfs/mut0511.pdf). One objective of the epidemiology overview was to attempt a quality ranking of studies.

10. The Committee noted the conclusion reached in the epidemiology overview that all of the studies were limited in design, particularly with regard to measurement of exposure, study size, the assessment of selection and recruitment biases. Many of the studies provided information on demographics, medical history, lifestyle factors, potential occupational exposures to materials other than pesticides (e.g. solvents, radiation), and also information on type of pesticides used, duration and frequency of exposure and use of protective measures. However these data had generally not been used in the analyses reported and the majority of papers did not provide a specific analysis of individual pesticides. It was noted that the majority of studies were not sufficiently large to allow an evaluation of all the variables for which data might be available. Study designs were generally cross sectional, although a few had taken multiple samples (e.g. at different time points in a growing season). The time interval between exposure and sampling thus varied considerably between studies and this might affect the conclusions which could be drawn. There were limitations in the statistical approaches used in many of the studies. Thus for example many did not consider the form of the population distribution and made unsubstantiated assumptions that it was normal . The reporting of modelling was variable and in most cases was not adequate. It was noted that the papers tended to focus on statistical significance even when the absolute difference between groups was tiny. Overall it was not possible to identify any particular study that was clearly better in design and reporting than the other papers in the 24 studies identified by COM.

11. The COM considered the epidemiology overview at its 26 May 2005 meeting. Members agreed that the review had highlighted and confirmed their views on the limitations of the data set. However members agreed to review the data in order to reach the most appropriate conclusions possible.

COM Review of selected studies.

12. The COM considered the full published reports and a narrative summary of the selected studies.
(http://www.advisorybodies.doh.gov.uk/pdfs/MUT0419.pdf
http://www.advisorybodies.doh.gov.uk/pdfs/mut051.pdf)

Criteria for evaluation

13. The COM undertook an evaluation of the control data from the 24 studies for micronuclei and chromosomal aberrations in peripheral blood lymphocytes. Such an evaluation might aid in the assessment of the data from studies and help to decide what magnitude of response was suggestive of a positive result. 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 as would be expected. Members felt the available data suggested that the distribution of chromosomal aberrations in human peripheral blood lymphocytes was consistent with an approximately binomial distribution, whilst distribution of micronuclei was much more dispersed than would be expected for binomial/Poisson distributions. It was considered that

there might be bimodal or trimodal distributions indicating possible subpopulation effects and that more data would help to resolve the actual distribution of these indices in peripheral blood lymphocytes. The large overall variation in the negative control (reference) data (approximately 16 fold for micronuclei and chromosomal aberrations) suggested that it was not possible to define a single historical control range. Members agreed that statistical significance from adequately conducted studies in combination with magnitude of response represented the most appropriate approach to evaluating the results of studies. Members agreed that the distribution of data should be assessed prior to consideration of the most appropriate statistical approach to analysis and that the effect of confounding factors should be clearly evaluated

14. The COM compared the magnitude of response seen in the 24 studies of pesticide applicators with that reported for patients undergoing treatment with cytostatic medicines and nurses occupationally exposed to these medicines. Members were surprised at the small magnitude of response in the biomonitoring studies of nurses or patients exposed to cytostatic medicines. The mean fold increase in nurses (1.8, range 1.5-2.2) and in patients (mean 2.1, range 1.5-2.7) derived from studies for either micronuclei or chromosomal aberrations was similar to that reported for pesticide applicators in the studies reviewed by COM (1.7, range 0.8-5). Members noted that the higher maximum fold increase in pesticide applicators compared to nurses or patients exposed to cytostatic medicines might reflect differences in the extent of control for confounding factors between studies. Members considered that the factors which accounted for the variance in the indices of genotoxicity in these biomonitoring studies (chromosome aberrations and micronuclei predominantly in circulating blood lymphocytes) in nurses and cancer patients exposed to cytostatic medicines had not been fully evaluated. The biomonitoring indices of genotoxicity were observed to differ considerably in the control populations of different studies. Members concluded, therefore, that it was not possible to establish a minimum fold increase for biological importance that could be applied to studies of pesticide applicators based on the studies in nurses and cancer patients exposed to cytostatic medicines. Further, it was concluded that factors affecting variance in genotoxicity indices used in the biomonitoring studies of pesticide applicators which had been reviewed were not understood adequately. In this respect members considered it would be very difficult to infer causality for the small magnitude responses seen in the biomonitoring studies of pesticide applicators. There was a need for more data on the background variability in the general population of biomonitoring indices of genotoxicity, and on factors affecting variance, which was required before a proper assessment of studies could be made.

15. Members agreed that consideration of statistical significance and magnitude of effects from adequately conducted studies was the most appropriate approach to evaluating the available data. Members confirmed that the available information was severely limited and hence no definite conclusions could be drawn with regard to any U.K. agricultural applications of pesticides or to individual pesticide active ingredients. However the COM

agreed that it would be appropriate to consider what conclusions could be derived from the selected studies.

Consideration of available data on exposure from selected studies

16. The Committee noted the limited information on exposure. The only direct exposure measurements were reported in the study by Garry et al [18] for exposure to 2,4-dichlorophenoxyacetic acid. Information had been provided on the identity of pesticides which applicators had used in 15 (11 reporting positive results and 4 reporting negative results) out of the 24 studies reviewed.[5,7,11,14-16,18,20,22,31,33,35,45,48,52] This information had been reviewed in the context of information from the Pesticide Usage Survey regarding information on use over the period from 1993 up to 2002 and also with regard to the available information on classification status under Directive EC/67/548 with regard to mutagenicity which had been provided by HSE. Members noted that apart from the study published by Garry et al [18] the magnitude of pesticide exposure in these studies had not been recorded and information on use of personal protective clothing had not been documented in many reports.

17. A number of the published papers selected by the COM had reported information which supported the view that the lack of protective clothing used by pesticide applicators/workers was associated with evidence of increased genotoxicity indices in biomonitoring studies.[11,15,22,32,45] It was noted that there was some limited evidence to suggest that work practices in green house (such as avoiding use of protective clothing in humid conditions and on re-entry after pesticide applications) might be potential sources of pesticide exposure.[32,61,82] It was also uncertain to what extent the application practices cited in the published reports were relevant to UK agricultural practice. In addition data from 2003 onwards on pesticide use was not available at the time of the COM consideration

18. Using information from the Pesticide Usage Survey, the amount of metam sodium and carbendazim increased over part of the period 1993-2001 in floricultural and green house applications. The area of outdoor bulbs and flowers sprayed with carbendazim was reported to increase. Increases in the use of bifenthrin, metam sodium and thiram were reported in agricultural practices. The Committee noted that with regard to pesticide active ingredients that were currently approved for use in the U.K. and which were also classified with regard to mutagenicity under EC/67/548 , carbendazim was used in 4/11 positive and 1/4 negative studies respectively [5,22,31,35,52]. This represented a very small and incomplete amount of information. It was noted that one of these studies had used micronucleus or chromosomal aberration analyses which could potentially be affected by spindle inhibitors such as benzimidazoles.[,31,] However it is unclear whether the results derived from the comet assay[22,35] could be related to benzimidazoles and there is no evidence for direct binding of benzimidazoles to DNA and hence the results of the remaining DNA-adduct study[52] were unlikely to be related to benzimidazole exposure. It was noted that there were a number of other classified mutagens listed in the positive studies which were not approved for use in the U.K.

19. Members considered the Bolognesi et al 2004 study in detail as this was the only available study with provided data on the specific aneuploidy inducing effects of benzimidazoles.[5] Members confirmed that this study should be considered as negative using the criteria agreed by the COM during the review of studies of pesticide applicators. Members agreed this was one of the better studies which had been considered as the investigators had attempted to use a specific index of exposures to tubulin inhibitors such as carbendazim and benomyl, although there were limitations in the dosimetric accuracy of the index of exposure, in the small number of individuals studied and in the lack of correction for multiple comparisons used in the statistical analysis. Members considered that the results of the centromere specific investigations were based on a very small number of individuals and no interpretation of biological significance could be placed on the data. The data provided in this study were consistent with the general conclusions on interpretation given below in paragraphs 22 and 23.

Consideration of data presented on duration of exposure

20. There were limited data on estimated duration of exposure to pesticides in 6 out of the 24 selected studies.[5,7,22,30,44,45]. Four of these studies reported a positive correlation between duration of exposure and increased indices of genotoxicity[7,22,44,45] whereas the remaining two studies reported a negative correlation.[5,30] The stratification into groups according to exposure was generally based on periods of 10 years or more. No rationale was given in the studies for the stratification of exposure groups according to duration of exposure. The magnitude of increased indices of genotoxicity with duration of exposure was small in all of the studies. The Committee was aware that biomarkers of genotoxicity (such as chromosome aberrations and micronuclei) increase in frequency with age and that this potential confounding factor had not been considered adequately in the analyses reported. The Committee agreed it was not possible to draw any definite conclusions based on these data, although it is noted that three out of four of the studies which reported on duration of exposure and which also reported a positive response had documented evidence for exposure to benzimidazole pesticides.

Consideration of use of Personal Protective Equipment (PPE)

21. None of the selected studies specifically investigated the effect of use of PPE on biomonitoring indices of genotoxic effects in pesticide applicators. The extent of PPE usage, where reported, varied considerably. Thus in some reports, no PPE was used,[29,30] whilst other reports describe conditions in which most pesticide applicators use PPE.[5,47] Several studies reported significant increases in chromosome aberrations and micronuclei correlated with a lack of PPE use during pesticide application.[11,15,31,33] It is noted that floriculturalists might report using PPE, but some investigators note that due to humid conditions within greenhouses appropriate PPE is not always worn. The Committee noted that a correlation between the lack of use of PPE and

increased biomonitoring indices of genotoxicity but concluded that no definite conclusions could be reached with regard to exposure to pesticides based on the available data.

COM Interpretation of available data

22. The Committee was aware of the guidance available for the conduct on biomonitoring studies of genotoxicity from Albertini et al [71]but agreed that having considered a large data set of studies on pesticide applicators that more research and guidance on the factors affecting the background variance of biomonitoring indices of genotoxicity was required before such studies could be fully interpreted particularly with regard to the significance of the small magnitude of response seen in the available studies

23. The COM discussed whether any proposed study should focus on an occupational group (such as floriculture) or on specific pesticides. Members acknowledged that the evidence was limited particularly with regard to the design, conduct, reporting and analysis of the available studies both with respect to identifying either an occupational category or specific pesticides. It was noted that a limitation in the available published literature concerned relevant information on the mutagenicity of mixtures of pesticides. Members were aware of the COT report on mixtures of pesticides (the WIGRAMP report http://www.food.gov.uk/science/ouradvisors/toxicity/COTwg/wigramp/).[83] The COT working group had identified benzimidazoles as a possible common mechanism group of compounds for further evaluation.
The Committee considered that although a UK study based on use of carbendazim in floriculture represented a reasonable proposal, there were considerable difficulties in undertaking and interpreting such a study. In particular it was agreed that more background information on the factors affecting the variance of biomonitoring indices of genotoxicity in unexposed populations would be required before a study of specific pesticide exposures was undertaken. If appropriate background information were available, then it was agreed that a longitudinal study where individuals acted as their own controls would be most appropriate. It was agreed that there was supporting evidence that biomonitoring for urinary excretion of 5-hydroxy-2-benzimidazole (5HBC) could be used to assess exposure and uptake of carbendazim.[84] Members commented that the application of Personal Protective Equipment (PPE) specified for use of carbendazim products (such as in floriculture) in the U.K. meant that it was unlikely that any increase in genotoxicity could be detected with any reliability. Any such study would have to be very large and there were doubts as to whether an appropriate exposure group could be identified.

COM conclusions

24. The Committee was aware that no DNA reactive *in-vivo* mutagens were used as active ingredients in approved formulated pesticide products in the U.K. It was noted that benzimidazoles were used in a number of approved pesticide products since a risk assessment could be undertaken for the

threshold related effects of these active ingredient on microtubule inhibition. The Committee confirmed that the available information was severely limited and hence no definite conclusions could be drawn with regard to any U.K. agricultural applications of pesticides or to individual pesticide active ingredients. The COM concluded;

i) The COM review was based on 70 retrieved published studies of biomonitoring of genotoxicity in pesticide applicators. The evidence covered a large number of types of applications and a wide diversity of pesticide mixtures. The COM selected 24 studies from which conclusions could be drawn through the application of a quality screen of retrieved studies. An independent epidemiological overview of these 24 studies reported that all had significant design and evaluation faults. The COM agreed that any conclusions reached on this evidence would be limited by the poor quality of the available studies.

ii) The COM agreed that following a review of the 24 selected studies there was limited evidence supporting increased biomonitoring indices of genotoxicity in biomonitoring studies of pesticide applicators. The Committee agreed that it was not possible to make any conclusions regarding exposure-response from the selected biomonitoring studies of genotoxicity because of the inadequacy and unreliability of exposure measurements and the generally small increases in response. The Committee noted that there was no published study of pesticide applicators using pesticide mixtures in the U.K.

iii) The Committee agreed that the factors which accounted for the variance in biomonitoring indices of genotoxicity (chromosome aberrations and micronuclei predominantly in circulating blood lymphocytes) in nurses and cancer patients exposed to cytostatic medicines and in pesticide applicators had not been fully evaluated. It was not possible to define a minimum increase in biomonitors of genotoxicity associated with cytostatic medicines from the available studies on nurses and cancer patients. Based on these observations and the large inter-study variation for the biomonitoring indices of genotoxicity in unexposed populations, the Committee concluded that it would be very difficult to infer causality for the small magnitude responses seen in the biomonitoring studies of pesticide applicators. There was a need for more data on the background variability in the general population of biomonitoring indices of genotoxicity, and on factors affecting variance, which was required before a proper assessment of studies could be made..

iv) The COM agreed that it is very difficult to draw conclusions on what might be the most appropriate biomonitoring study for U.K. pesticide applicators. There was some very limited evidence to suggest that an appropriate study of floriculturalists using benzimidazoles (e.g carbendazim) might represent a reasonable

proposal. The Committee concluded that in view of the large numbers of individuals which would be required in order to detect an effect in such a study it was most unlikely that a sufficiently large and appropriate exposure group could be identified.

October 2005.

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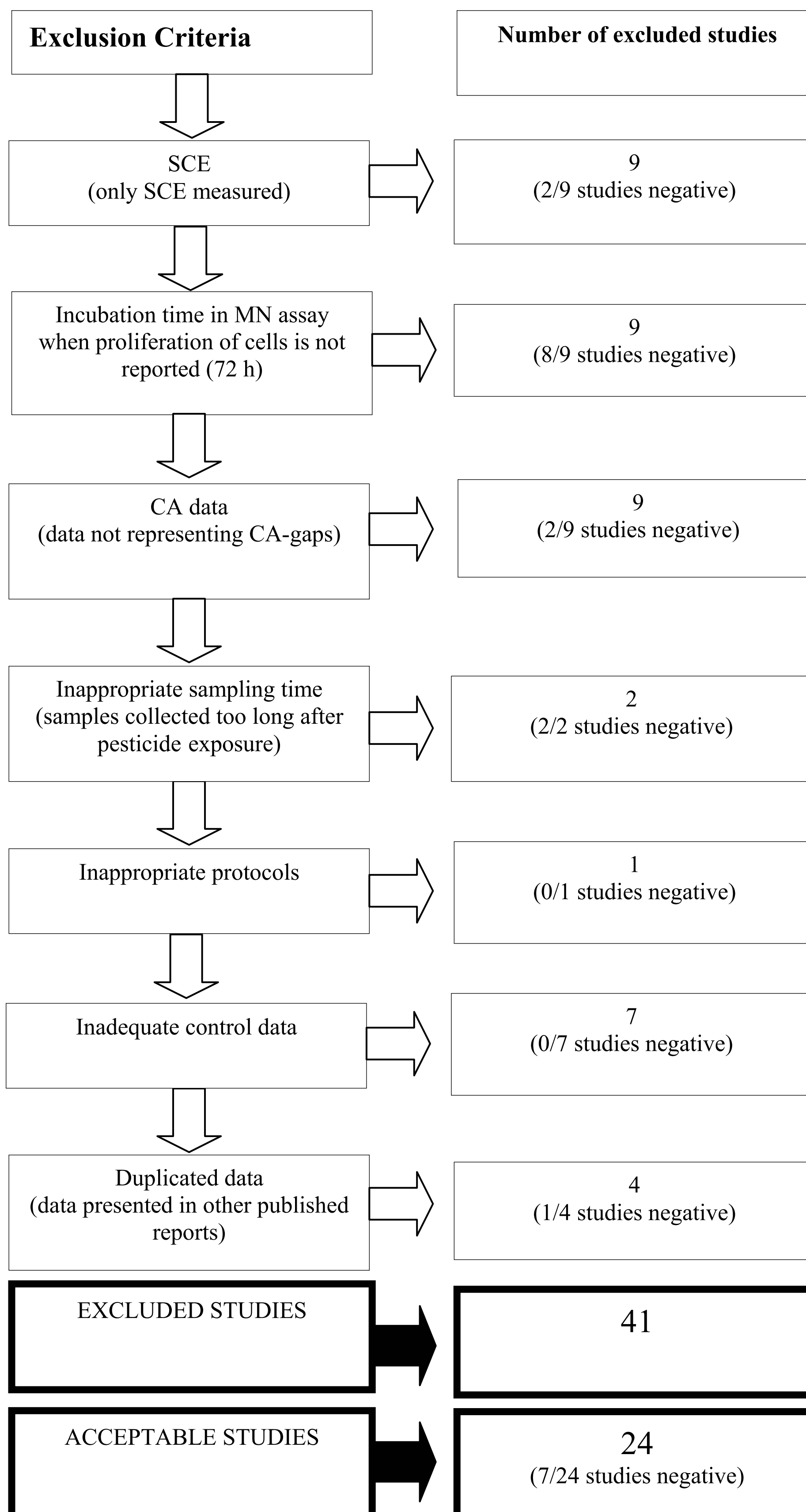
ANNEX 1 TO DRAFT WORKING PAPER ON BIOMONITORING STUDIES OF GENOTOXICITY IN PESTICIDE APPLICATORS JULY 2005

Flow chart of excluded studies.

The flow chart demonstrates how many studies were excluded from further analysis due to various selection criteria, and the data reported in such studies.



ANNEX 2 TO COM STATEMENT ON BIOMONITORING STUDIES OF GENOTOXICITY IN PESTICIDE APPLICATORS:

Data reported in selected studies

Author	Results	Data for controls	Data for exposed subjects	Fold increase over controls
[a]Bolognesi *et al.*, 1993b	+ MN	MN frequency = 6.67 ± 3.12	MN frequency = 8.57 ± 5.02 (mean / 1000 cells ± SD) RR = 1.25; 95 % CI = 1.11 – 1.41	**1.3-fold increase**
[a]Bolognesi *et al.*, 2004	- MN	Total MN (C+MN) = 2.18 ± 6.31 Total MN (C-MN) = 1.32 ± 3.38	Total MN (C+MN) = 2.79 ± 12.21 Total MN (C-MN) = 1.56 ± 6.00 (mean / 1000 cells ± SD)	**1.2-fold increase** **1.3-fold increase**
[a]Carbonell *et al.*, 1995	+ CA	Spring/summer Cells with aberrations = 4.56 ± 2.53 % Chromatid-type aberrations = 3.14 ± 2.76 % Chromosome-type aberrations = 1.90 ± 1.51 % Total aberrations = 5.04 ± 2.85 % Autumn/winter Cells with aberrations = 3.39 ± 2.4 % chromatid-type aberrations = 2.57 ± 2.0 % Chromosome-type aberrations = 1.32 ± 1.96 % Total aberrations = 3.90 ± 3.23 %	Spring/summer Cells with aberrations = 6.27 ± 2.96% Chromatid-type aberrations = 5.31 ± 3.12 % Chromosome-type aberrations = 1.63 ± 1.56 % Total aberrations = 6.93 ± 3.5 % Autumn/winter Cells with aberrations = 3.69 ± 2.14 % chromatid-type aberrations = 2.49 ± 0.56 % Chromosome-type aberrations = 1.21 ± 1.83 % Total aberrations = 3.70 ± 2.15 % (mean ± SD)	1.4-fold increase 1.7-fold increase 0.9-fold increase **1.4-fold increase** 1.1-fold increase 1.0-fold increase 0.9-fold increase **1.0-fold increase**
[a]De Ferrari *et al.*, 1991	+ CA + SCE	Chromatid-type aberrations = 4.44 ± 3.06 Chromosome-type aberrations = 1.08 ± 1.28 Complex rearrangements = <0.02 Total aberrations = 5.52 ± 4.12	Exposed subjects Chromatid-type aberrations = 7.46 ± 6.22 Chromosome-type aberrations = 2.72 ± 1.58 Complex rearrangements = 0.12 ± 0.12 Total aberrations = 10.30 ± 7.18 Exposed subjects with bladder cancer; Chromatid-type aberrations = 5.07± 3.90 Chromosome-type aberrations = 2.65 ± 02.26 Complex rearrangements = 0.30 ± 0.62 Total aberrations = 8.02 ± 4.98 (Mean / 100 metaphases ± SD)	1.7-fold increase 2.5-fold increase 6.0-fold increase **1.9-fold increase** 1.1-fold increase 2.5-fold increase 15.0-fold

				increase **1.5-fold increase**
[b]Dulout *et al*., 1985	+ CA	Abnormal cells = 2.65 ± 1.01 % Gaps = 1.56 ± 2.09 Chromatid breaks = 1.70 ± 0.74 Chromosome breaks = 0.54 ± 0.62 Dicentric chromosome and ring chromosome = 0.10 ± 0.21	Abnormal cells = 2.71 ± 0.36 % Gaps = 2.43 ± 1.62 Chromatid breaks = 1.51 ± 1.26 Chromosome breaks = 0.95 ± 1.08 Dicentric chromosome and ring chromosome = 0.43 ± 0.84 (CA / 100 cells - gaps ± SD)	**1.0-fold increase** **1.6-fold increase** **0.9-fold increase** **1.8-fold increase** **4.3-fold increase**
[a]Falck *et al*., 1999	+ MN	MN frequency 0.5 µg/ml BrdU = 7.4 ± 3.1 1 µg/ml BrdU = 7.4 ± 3.1	0.5 µg/ml BrdU = 7.8 ± 2.4 1 µg/ml BrdU = 8.0 ± 2.7 (mean / 1000 cells ± SD)	**1.1-fold increase** **1.1-fold increase**
[b]Garry *et al*., 1996	+ CA	Rearrangement frequency = 0.4 ± 0.57	Rearrangement frequency Fumigant = 1.4 ± 1.44 Insecticide = 1.4 ± 1.28 Herbicide = 1.0 ± 1.34 (mean ± SD)	**3.5-fold increase** **3.5-fold increase** **2.5-fold increase**
[b]Garry *et al*., 2001	+ CA	Translocations/inversions/deletions = 0.65 ± 1.12	Translocations/inversions/deletions Low volume (1-100 gall) = 1.20 ± 1.13 Mid-range (100-1000 gall)= 1.00 ± 1.13 Heavy (>1000 gall) = 2.22 ± 1.14	**1.9-fold increase** **1.5-fold increase** **3.4-fold increase**
[b]Gomez-Arroyo *et al*., 2000	+ MN	MN frequency = 0.38 ± 0.021	MN frequency = 1.01 ± 0.03 (mean / 100 cells ± SD)	**2.7-fold increase**
[b]Grover, *et al.*, 2003	+ comet	Smokers Comet tail length = 7.03 ± 11.46 Non-smokers Comet tail length = 10.34 ± 13.25	Smokers Comet tail length = 18.26 ± 9.76 Non-smokers Comet tail length = 19.75 ± 14.48	**2.6-fold increase** **1.9-fold increase**
[b]Hogstedt et al., 1980	- CA	Cell with aberrations = 4.6 %	Cell with aberrations = 4.2 %	**0.9 -fold increase**
[a]Kourakis *et al*., 1992	+ CA	Chromosome-type aberrations = 0.2 ± 0.37 chromatid-type aberrations = 0.34 ± 0.60 Total aberrations = 0.54 ± 0.90 %	Chromosome-type aberrations = 1.34 ± 1.62 chromatid-type aberrations = 0.80 ± 0.81 Total aberrations = 2.14 ± 1.62 % (mean / 100 metaphases ± SD)	4.6-fold increase 6.1-fold increase **5.0-fold increase**

[a]Lander *et al.*, 2000	+ CA	Pre-season Chromatid-type aberrations-gaps = 1.03 ± 0.82 Chromosome-type aberrations-gaps = 0.28 ± 0.45 Total aberrations-gaps = 1.31 ± 0.85 %	Pre-season; Chromatid-type aberrations-gaps = 0.87 ± 0.95 Chromosome-type aberrations-gaps = 0.45 ± 0.74 Total aberrations-gaps = 1.32 ± 1.23 % Post-season Chromatid-type aberrations-gaps = 1.04 ± 0.99 Chromosome-type aberrations-gaps = 0.34 ± 0.56 Total aberrations-gaps = 1.37 ± 1.20 % (Mean / 100 metaphases ± SD)	0.84-fold increase 1.6-fold increase **1.0-fold increase** 1.2 (1.0) -fold increase 0.8 (1.2) -fold increase **1.0 (1.0)** -fold increase compared with pre-season (compared with controls)
[a]Lebailly *et al.*, 1998	+ comet	Beginning of spraying season = 30 Beginning of spraying season = 30	Middle of spraying season = 43 End of spraying season = 36	**1.4-fold increase** **1.2-fold increase**
[a]Lebailly *et al.*, 1998b	+ comet	DNA damage Before spraying Mixture of pesticides = 48 % Herbicides on wheat = 30 % Fungicides on wheat = 43 % Fungicides & insecticides on peas = 36% Tail moment Before spraying Mixture of pesticides = 3.21 Herbicides on wheat = 2.30 Fungicides on wheat = 3.64 Fungicides & insecticides on peas = 2.39	DNA damage After spraying Mixture of pesticides = 56 % Herbicides on wheat = 28 % Fungicides on wheat = 35 % Fungicides & insecticides on peas = 39% Tail moment After spraying Mixture of pesticides = 3.92 Herbicides on wheat = 1.93 Fungicides on wheat = 3.58 Fungicides & insecticides on peas = 3.16 (mean)	**1.2-fold increase** **0.9-fold increase** **0.8-fold increase** **1.1-fold increase** **1.2-fold increase** **0.8-fold increase** **0.9-fold increase** **1.3-fold increase**
[a]Lebailly *et al.*, 2003	- comet	DNA damage Morning before pesticide use = 10 % (2-21 %) Tail moment Morning before pesticide use = 4.35 ± 1.11 (2.16-5.85)	DNA damage Evening after pesticide use = not measured Following morning = 13 % (5-49%) Tail moment Evening after pesticide use = not measured Following morning = 4.80 ± 2.57 (3.18-12.76) (Mean ± SD)	**1.3 -fold increase** (compared to before pesticide use) **1.1-fold increase** (compared to before pesticide use)

[a]Munnia *et al*., 1999	+ DNA adducts	DNA adducts = 2.17 x 10^9 ± 5.75 RAL	DNA adducts = 8.50 x 10^9 ± 14.95 RAL (Mean ± SD)	**3.9-fold increase**
[a]Mustonen *et al*., 1986	- CA	Aberrant metaphases-gaps Non-smokers; 1.5 ± 0.73 Smokers; 1.9 ± 1.2	Aberrant metaphases-gaps Non-smokers; 1.2 ± 1.5 Smokers; 1.8 ± 1.26 (Mean ± SD)	**0.8-fold increase** **1.0-fold increase**
[b]Paldy *et al*., 1987	+ CA	Chromosome aberrations = 1.1 ± 0.36	Years of exposure Chromosome aberrations 0-5 years = 2.96 ± 0.36 6-10 years = 3.55 ± 0.75 11-15 years = 4.28 ± 0.76 (Sum of aberrations / 100 cells without gaps ± SD)	**2.7-fold increase** **3.2-fold increase** **3.9-fold increase**
[a]Pasquini *et al*., 1996	+ MN	MN frequency = 13.30 ± 5.35 Overall MN frequency = 13.30 ± 5.35	MN frequency (>19 year) = 18.30 ± 7.22 Overall MN frequency = 15.98 ± 7.65 (Mean / 1000 cells ± SD)	1.37-fold increase **1.2-fold increase**
[a]Pastor *et al*., 2001b	- MN	MN frequency = 16.38 ± 12.19	MN frequency = 12.20 ± 6.58	**0.7-fold increase**
[a]Pastor *et al*., 2002a	- MN	MN frequency = 10.3 ± 7.06	MN frequency = 10.22 ± 7.06	**1.0-fold increase**
[a]Peluso *et al*., 1996	+ DNA adducts	DNA adducts = 9	DNA adducts = 42 [44]	**4.7-fold increase**
[a]Piperakis *et al*., 2003	- comet	DNA damage Male non-smokers = 82.3 ± 14.1 Female non-smokers = 81.1 ± 16.12	DNA damage Male non-smokers = 83.2 ± 14.02 Female non-smokers = 82.1 ± 13.14 (mean ± SD)	**1.0-fold increase** **1.0-fold increase**

[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.