

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

BENZIMIDAZOLES: AN APPROACH TO DEFINING A COMMON ANEUGENIC GROUPING

Background

1. The purpose of this COM review is to consider whether a number of benzimidazoles, compounds used as pesticides or veterinary medicines, should be grouped based on a common mechanism of toxicological (genotoxic) action, and to consider the data requirements for including or excluding a chemical from the group. The COM considered papers at its May 2006 and October 2006 meetings. This paper contains a revised proposal for a decision tree, which could be used to determine whether a compound was included or excluded from a benzimidazole common mechanism group.

2. The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) has recommended that the default assumptions in risk assessments of combined exposure should be that chemicals with different toxic action will act independently, and that those with the same toxic action will act additively (COT, 2002). The COT has observed that where groups of chemicals with similar modes of action have been studied, additivity (or less than additivity) has been demonstrated rather than synergy. Examples include endocrine disrupters that act by agonism of oestrogen receptors, dioxin-like compounds and organophosphates (COT/COM/COC, 2004).

3. New EU pesticide legislation to be introduced in the next couple of years will require consideration of combination effects in the approval process (it is termed cumulative risk assessment, but refers to exposure to multiple residues rather than bioaccumulation of a single substance in the body). Current indications are that it is likely that there will be one of three outcomes to an assessment:

- i) A substance is considered to act independently when exposure is below the reference dose, i.e. acceptable exposure would not result in any interaction or combination effects with other pesticide residues that would be harmful to health.
- ii) If a group of substances can be assigned to a "common mechanism group", then they would be regulated as a group, with an assumption of dose additivity.
- iii) A substance could have interactions resulting in synergy with other substances. In this case, either it would not be approved or it would be necessary to estimate the potential degree of

synergy which would be taken into account by additional uncertainty factors in order to protect public health.

Common mechanism groups and dose additivity

4. Dose additivity is also known as simple similar action, or simple joint action, and occurs over the whole dose-response curve. As stated above, this has been demonstrated for some groups of similarly acting chemicals. The effect of a mixture is obtained by summing the doses of the individual compounds, after adjustment for differences in their potencies (COT/COM/COC, 2004).

5. An example of a use of the common mechanism approach is the risk assessment of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and dioxin-like polychlorinated biphenyls, environmental contaminants widely present in food. These compounds are assessed as a group based on the concept of dose addition, since they exert common toxic effects due to actions on the aryl hydrocarbon (Ah) receptor. Toxic equivalency factors (TEFs) have been established by the WHO for each congener to account for differences in potency. The COT has recognised that the assumption of additivity, and the allocation of TEFs, for these compounds involves a pragmatic process because of the limitations in the scientific basis.

6. The COT has noted that not all compounds which interact with the Ah receptor are agonists, but considered that if partial agonism was the concern, an assumption of additivity would make the risk assessment over-protective rather than under-protective (COT/COC/COM, 2004).

7. If a benzimidazole common mechanism group is formed, subsequent steps will require consideration of the toxic effects observed in toxicological studies, the relation of the observed effects to mutagenicity, and the in vivo potency of these compounds.

Definition of “common mechanism” of toxicity

8. The COT report did not specifically define “common mechanism” in its 2002 report. However, it variously referred to chemicals which would be in a common mechanism group as “similarly acting”, having “the same” toxic action, or having a “common mode of action” (COT, 2002). The COT report recommended that groups of pesticides having common targets of toxicological action should be identified. Such work might include the identification of sites of action at a molecular level, to identify those compounds that would be expected to show simple similar action. The COT recommended that studies of protein and/or RNA expression, or of enzyme or hormonal activity, may be appropriate in some cases.

9. The US Environmental Protection Agency (EPA), which is required by the 1996 Food Quality Protection Act in the USA to assess risks from exposure to combinations of pesticides, has defined a common mechanism group as consisting of “Two or more chemicals or other substances that

cause a common toxic effect(s) by the same, or essentially the same, sequence of major biochemical events (i.e., interpreted as mode of action).” (http://www.epa.gov/pesticides/glossary/index.html#common_mechanism, accessed 27 November 2006).

10. Members will wish to consider how broad or narrow the definition of a common mechanism for certain benzimidazoles needs to be.

Risk assessment of individual benzimidazoles

11. The COM has previously advised that it is reasonable to assume that aneuploidy-inducing chemicals (particularly those that function by damaging the cell division apparatus) have a threshold of action. In 1993 the COM provided advice on methodology for identifying thresholds for aneugens acting by spindle inhibition. In 1996 the COM considered studies on the benzimidazoles carbendazim and benomyl and advised on threshold concentrations for these aneugenic substances (COT/COC/COM, 1996). In 1997 the COM saw similar data for thiophanate-methyl. The former EU Scientific Committee on Plants (SCP) has produced similar conclusions on carbendazim, benomyl and thiophanate-methyl, concluding that since multiple copies of tubulin molecules are present in proliferating cells, in the presence of low concentrations a limited number of tubulin molecules will be affected and consequently no toxicological adverse effects will ensue (SCP, 2001). Other committees have drawn similar conclusions for other benzimidazoles where genotoxicity data have provided evidence for aneugenicity.

Revised decision tree proposal

12. Based on comments Members made at the October 2006 meeting and subsequently, a revised draft decision tree has been produced for Members' consideration (Annex A).

13. One comment received was that a chemical may share a structural similarity to aneugenic substances but be a direct acting mutagen, or a chemical that is an aneugen may additionally be mutagenic by a direct DNA-acting mechanism. The decision tree has been revised to clarify that substances should not be assumed to be only aneugens unless the mutagenicity data are sufficient to demonstrate that mutagenic effects observed are solely via this mode of action.

14. Based on one comment that a consideration of functional effects on tubulin is more valuable than detailed information on binding sites, the revised decision tree requires evidence for a common effect on tubulin in addition to evidence for aneugenicity, rather than requiring evidence of binding to tubulin at the same binding site.

15. Members also commented on the possibility of combined effects other than dose addition, including synergy, sub-additivity and antagonism. As noted by the COT, if partial agonism is a concern then an assumption of dose additivity will make a combined risk assessment overprotective rather than

underprotective. In order to exclude the possibility of synergy, and to test the assumption of dose additivity, a small number (e.g. 3) of benzimidazoles assigned using this decision tree to a common mechanism group could be studied individually and in various combinations *in vitro* at concentrations relevant to human exposure. In principle, any benzimidazoles which pass through the decision tree into a common mechanism group should be suitable for use in such a study. Members will wish to consider whether they agree with this suggestion, and what type of study would be most appropriate. A footnote has been added to the decision tree recommending that such studies be performed.

16. There is also a need to consider the effects of combined exposure to benzimidazoles and other aneugenic substances. It is suggested that non-benzimidazoles may be added to the common mechanism group if dose addition is demonstrated with at least one benzimidazole in the common mechanism group.

Questions to the Committee

17. The Committee is invited to consider the following questions and the revised proposed decision tree at Annex A.

- i) Do Members agree with the revised proposed decision tree? Are any amendments required?
- ii) Is there a rationale for considering that the benzimidazoles may produce greater effects in combination than would be expected on the basis of dose addition (i.e. synergy) at doses relevant to human exposure, i.e. within ADIs for individual chemicals? Do Members agree with the suggestion of conducting mixture studies for a small number of benzimidazole compounds assigned to a common mechanism group using the decision tree in order to test the default assumption of dose additivity? What type of study would Members recommend?
- iii) Do Members agree with the suggestion for how other aneugens which are not benzimidazoles may be added to the common mechanism group?

**Secretariat
January 2007**

References

COT/COM/COC (2004). Committees of Toxicity, Carcinogenicity and Mutagenicity of Chemicals in Food, Consumer Products and the Environment, Annual Report 2004. pp 15-18: Mixtures of food contaminants and additives. Available at:

<http://www.food.gov.uk/science/ouradvisors/toxicity/reports/cotcomcocrep2004>

COT (2006). Statement on organic chlorinated and brominated contaminants in shellfish, farmed and wild fish. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment. Statement 2006/06. Available at:

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SCP (2001). Opinion of the Scientific Committee on Plants regarding the evaluation of Benomyl, Carbendazim and Thiophanate-Methyl in the context of Council Directive 91/414/EEC concerning the placing of plant protection products on the market (opinion adopted by the Scientific Committee on Plants on 7 March 2001). Available at:

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Revised draft proposal for a decision tree for deciding when to include a compound in a benzimidazole common mechanism group.

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Structure contains a benzimidazole ring



Evidence that compound is not a DNA-reactive mutagen^a



Evidence that compound is aneugenic either in vitro (e.g. micronuclei containing whole chromosomes, polyploidy, inhibition of mitosis) or in vivo (e.g. positive micronucleus assay with centromere positive staining). Negative in vivo results will overrule other positive results only if acceptable kinetic data are available to indicate sufficient exposure of the target tissue



A rationale for considering that aneugenicity is due to a common functional effect, i.e. reduced tubulin polymerisation by either inhibition of tubulin polymerisation or depolymerisation^b



Add to common mechanism group^{c,d}

Notes:

^a In some instances there may additionally be a need to assess risks from combined exposure with other benzimidazoles which are aneugens even if the compound may also be a direct acting mutagen, in which case the substance may continue through the procedure.

^b Any *in vitro* data showing reduced polymerisation of mammalian tubulin would be suitable

^c Mixtures of selected benzimidazoles assigned to the common mechanism group should be studied *in vitro* at concentrations relevant to human exposures in order to test the default assumption of dose additivity and exclude the possibility of greater than additive effects (i.e. synergy).

^d Non-benzimidazoles which are considered to be aneugens may also be added to the common mechanism group if dose addition with at least one benzimidazole in the common mechanism group is demonstrated.