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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

JAN 27 1994

MEMORANDUM

SUBJECT: Polyhexamethylene Biguanide (PHMB; Baquacil): Review of Dietary Analysis Data Submitted by the Registrant.

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Review Section I, Toxicology Branch II
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and

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Registrant: Zeneca Biocides, Wilmington, Delaware

Action Requested: Review of dietary analysis data for PHMB submitted by the registrant in response to concerns raised by Toxicology Branch II, Health Effects Division.

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Data Summary:

In previous correspondence from the registrant, it was proven that extraction of PHMB from the diet for measurement of concentration of active ingredient was not possible due to strong binding of PHMB to dietary matrix. Drinking water studies were then initiated with PHMB in part due to the ability to provide analytical support for the concentration of PHMB used. However, it was later determined that the drinking water route was not an appropriate route of dosing, and that the dietary route should be used for long term toxicology studies with PHMB. Following a meeting between the registrant and representatives of HED, Toxicology Branch II, to discuss problems with analysis of test diets for PHMB concentration and homogeneity, it was determined that demonstration of the concentration of PHMB in dietary mixtures could be accomplished through gravimetric analysis, while homogeneity could be demonstrated through use of a dye with similar binding properties as PHMB. The data in the present submission address these issues.

Demonstration of PHMB Concentration in Dietary Mixtures

Diets for use in toxicology studies were prepared using CT1 diet (Special Diets Services Limited, Stepfield, Witham, Essex, UK), but Laboratory Diet A from the same supplier was used for preparing dietary premixes, as it accepts aqueous formulations more readily. Laboratory Diet A is intended for use in non-rodents, but according to the registrant, the differences in composition between CT1 and Laboratory Diet A are not marked. In addition, the quantity of Laboratory Diet A incorporated into the dietary mixture was considered to have little effect on the intended composition of a diet formulated for rodents.

Diets were prepared in 30kg or 60kg batches. Premixes of 1kg were prepared, with no more than 150ml of test substance (20% PHMB stock solution) added per premix. The required amount of test substance was added to 500g Laboratory Diet A and mixed with a mortar and pestle. The bottle containing PHMB was rinsed with 20ml deionized water, and this added as well to the premix. The mixture was passed through a 1mm sieve and any lumps ground down using a mortar and pestle and returned to the mixture. The premix was then mixed in a Kenwood Chef mixer for 10 minutes, after which a total of 500g CT1 diet (in 2 250g batches) was added to give a 1kg dry premix. This premix was then combined with the appropriate amount of CT1 diet in a Fielder Blender to give a total of 30kg or 60kg diet mixture.

Diet mixtures were stored at room temperature and used within 6 weeks of preparation, based on previous data showing stability of PHMB in deionized water for 6 weeks.

In reference to the long term dietary toxicity studies, the registrant provided 2 study numbers PRO936 and PMO937. These appear to refer to mouse and rat carcinogenicity/chronic toxicity studies, which can be inferred from the Phase IV review of PHMB which listed these as outstanding data requirements. In these studies, 4 dose groups, including control, were used. For study # PMO937, concentrations of 0, 400, 1200, and 4000 ppm PHMB were used and diets were made in 30kg batches. For study # PRO936, concentrations of 0, 200, 600, and 2000 ppm PHMB were used, and

diets were prepared in 60kg batches.

The following Tables from the report illustrate the nominal amount of test material which should be incorporated into the dietary mixtures to give the desired concentration in parts per million. The mass of test substance listed has taken into account the purity of the technical material (20.2% w/w).

TABLE 1A

Group	Dietary Concentration of PHMB (ppm, w/w)	Quantity of Test Substance to Prepare 30kg of Diet (g)	Colour Code
1	0	0	Blue
2	400	59.41	Green
3	1200	178.22	Yellow (Gold)
4	4000	594.06	Red

TABLE 1B

Group	Dietary Concentration of PHMB (ppm, w/w)	Quantity of Test Substance to Prepare 60kg of Diet (g)	Colour Code
1	0	0	Blue
2	200	59.41	Green
3	600	178.22	Yellow (Gold)
4	2000	594.06	Red

For test group 2, a single dietary premix containing 59.41g test material was used. For test group 3, two premixes containing 89.11g test material were used, and for test group 4, four premixes containing 148.5g test material were used. These weights of test material were supported by data provided in the report showing weights of test substance issued for preparation of dietary premixes.

Demonstration of Homogeneity of PHMB in Test Diets

Demonstration of satisfactory mixing of PHMB in the diet was achieved by use of methyl violet dye. For this determination, triplicate 10g portions of test diets (without PHMB) were weighed into tared 250ml conical flasks. A 2% nominal concentration of methyl violet was prepared by adding 20g methyl violet to 1000ml distilled water and

stirring until all dye that is reasonably practicable was dissolved. This solution was then filtered to give a clear blue solution. An appropriate amount of this solution was then added to weighed aliquots of test diets (10g) and mixed. For measurement of distribution of test diet, the methyl violet-test diet mixture was subjected to solvent extraction (100ml methanol) from the top, middle, and bottom of each test diet mixture and the resulting concentration of methyl violet measured by UV spectrophotometry Data for this analysis, as presented in Table 4 of the report, pages 17-23, show that the expected concentration of methyl violet was within 12% of nominal for all areas of test diet sampled (top, middle, and bottom). Based on the similar binding characteristics of methyl violet and PHMB, these data show that PHMB appears to be uniformly mixed in test diets using the procedures outlined in the present report.

The acceptability of the above analyses conducted by the registrant addresses an additional issue previously raised by the registrant. In a memo dated July 22, 1992 from Timothy F. McMahon, Toxicology Branch II, to Linda Deluise, Special Review and Reregistration, the request for upgrading of a rat teratology study conducted with PHMB (MRID # 65131) was denied pending review of dietary analysis data. Based on the review of the dietary analysis data as discussed above, the rat teratology study can be upgraded to core guideline data.