BASF

The Chemical Company

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Dr. Barbara Shane
Executive Secretary for the NTP BSC
NTP Office of Liaison, Policy and Review
NIEHS
P.O. Box 12233
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Research Triangle Park, NC

Dear Dr. Shane,

BASF Corporation is submitting comments to the Board of Scientific Counselors in response to the proposal to conduct reproductive and long-term studies of 2-ethylhexyl p-methoxycinnamate (CAS 5466-77-3; EHMC; OMC). This substance is used as a UV absorber for sunscreen application. As such, it has been used for many years without any apparent adverse effects in humans. More importantly, the database for OMC is rich with studies addressing virtually all concerns including endocrine disruption and the potential to produce carcinogenicity. In spite of the information currently available for OMC, the NTP has proposed conducting toxicokinetic, reproductive toxicity, and carcinogenicity studies. BASF Corporation finds the rationale of these studies to be weak. For the reproductive toxicity study, replicating the currently available study is unwarranted and is a misuse of animals. No new information will be obtained given the study design that was used for the reproductive toxicity study, and the high no-observed-effect level. Furthermore, BASF Corporation has additional unpublished data that it will make available to regulatory bodies that are concerned with assessing the risk fro endocrine disruption from exposure to OMC; these studies address some of the concerns voiced in the rationale. Summaries of these studies will be provided to NTP. For cancer, OMC is considered non-genotoxic and has been shown to protect against photocarcinogenicity. It seems unlikely that this material would be a cancer hazard in its own right.

BASF Corporation provides these comments to the Board in the hope that they will demonstrate that OMC is not a good candidate for further testing, and that the proposed studies should be reconsidered.

Regards,

Raymond M. David

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Manager, Toxicology

Comments to BSC on Proposed Studies of
2-Ethylhexyl *p*-methoxycinnamate

EXECUTIVE SUMMARY

- **Impact of nanoparticles encapsulation will reduce absorption not enhance it.** Based on the study published by Jimenez *et al* (2004), less EHMC is released into the stratum corneum from nanoencapsules than from without encapsulation. Therefore, nanoencapsulation is not a valid rationale for the study of systemic effects of EHMC.

- **Specific studies for further clarification of putative reproductive effects confirmed the absence of endocrine disruptive properties of EHMC.** A uterotrophic and Hershberger assay, as well as specific hormonal investigations in an oral repeated dose 28 day study have been conducted by BASF. Both studies indicate that EHMC is not an endocrine disruptor: no increase in uterine weight was observed in a standard guideline urterotrophic assay; and no treatment-related changes in hormone levels were observed after 28 days. Therefore, additional studies to clarify endocrine disruption are not warranted. In addition, conducting a Continuous Breeding study of EHMC would not provide useful additional information if there is no evidence of endocrine disruption and the NOAEL is 450 mg/kg from Schneider *et al.*

- **2-Ethyhexyl methoxycinnamate is not genotoxic and does not produce promote initiated cells.** The available photogenotoxicity studies do not indicate a photomutagenic effect by a toxic metabolite, free radicals or by degradation products. There was no evidence of a photocarcinogenic effect; rather EHMC delayed the median latent period for tumor development. Therefore, EHMC is not good candidate for carcinogenicity testing.

Comments:

- **Impact of nanoparticles encapsulation will reduce absorption not enhance it.** In its Research Concept review of the data for EHMC, NTP states:

> "Recently, new carrier systems like nanoparticle encapsulation and nanoemulsions have been investigated for enhancement of EHMC photostability and increased penetration into the stratum corneum compared to conventional oil/water emulsions."

This statement misrepresents the available information on the impact of nanoencapsulation. Based on the study published by Jimenez *et al* (2004[1]), less EHMC[2] is released into the stratum corneum from nanoencapsules than from without encapsulation. Jimenez *et al.* state that EHMC "remained primarily on the skin surface" with greater amounts on the surface from the encapsulated form than from the other vehicles (82-90% for nanocapsulated versus 48-60% for oil-in-water emulsions alone). A similar disparity was observed within the stratum corneum (8-18% for nanocapsulated versus 36-46% for oil-in-water emulsions). These conclusions are reflected in the Background document prepared by NTP:

> "In another *in vitro* study of octyl methoxycinnamate absorption through pig skin, considerably greater amounts of this chemical were absorbed when the free chemical was administered in emulsions than when the material was microencapsulated (Jimenez *et al.*, 2004)."

Therefore, if nanoencapsulation is used for EHMC, the likely outcome -- in fact, the intended purpose -- is to restrict the penetration of the UV absorbed and to keep as much as possible in the upper layers of skin. The data from Jimenez *et al* provide clear evidence of this phenomenon. Therefore, nanoencapsulation is not a valid rationale for the study of systemic effects of EHMC.

- **Specific studies for further clarification of putative reproductive effects confirmed the absence of endocrine disruptive properties of EHMC.**

In its Research Concept review for EHMC, NTP states:

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[1] Jimenez, M.M., Pelletier, J., Bobin, M.F. & Martini, M.C. (2004) Influence of encapsulation on the *in vitro* percutaneous absorption of octyl methoxycinnamate. *Int. J. Pharmaceutics*, **272** (1-2), 45-55.

[2] Jimenez et al refer to octyl methoxycinnamate in the text which they indicate is 2-ethyhexyl methoxycinnamate.

"These endocrine-related alterations and the controversy surrounding the effects indicate that further investigation and clarification of the reproductive effects of this potentially endocrine active compound is warranted."

BASF disagrees. Specific studies addressing androgenic/estrogenic properties, i.e. an uterotrophic and Hershberger assay, and specific hormonal investigations in an oral repeated dose 28 day study, have been conducted by BASF. In the uterotrophic assay, juvenile Wistar rats were treated with doses of 250 and 1000 mg/kg bw/day EHMC. In the high dose group, mean body weight gain was statistically significantly reduced (days 0-3: about 27% below the control value), whereas no significant changes in absolute and relative uterus weights and no differences in histology has been observed compared to control animals. Therefore, using this standard protocol, EHMC is not estrogenic.

Furthermore anti-androgenic efficacy was addressed in the Hershberger assay, dosing castrated and testosterone propionate (TP) treated Wistar rats with 300 and 1000 mg/kg bw/day EHMC. A slight but significant reduction of mean absolute ventral prostate weights has been observed, which could not be confirmed with the respective relative weights and a histopathological correlate.

Treatment			
EHMC (mg/kg bw/ day)	TP (mg/kg bw/ day)	Mean absolute ventral prostate weights (mg)	Standard deviation
0	0	18.917	1.425
0	0.4	107.55	10.294
300	0.4	93.25	9.987
1000	0.4	81.133	14.692

These findings are considered to be secondary to the slight terminal body weight reduction observed (-2.3% and -5.5% for 300 and 1000 mg/kg body weight EHMC). Furthermore, the weights of the other accessory sex organs were not significantly different from control animals. In line, no significant changes in serum testosterone, dihydrotestosterone and luteinizing hormone concentrations were found in the EHMC high dose group compared to the respective control.

Repeated dietary administration of EHMC (906 mg/kg body weight/day) over a period of 4 weeks showed slight decreases in food consumption and body weights. No changes in ovary weights and in estrus cycle duration were found and neither prolactin, follicle-stimulating hormone, luteinizing hormone, estradiol or progesterone serum levels were statistically significantly altered. Concerning thyroid hormones,

triiodothyronine (T3) and thyroid-stimulating hormone concentration were found to be unaffected by EHMC treatment whereas thyroxine (T4) was slightly but significantly elevated (44.77 ±7.94 nmol/l in control group versus 53.43 ±4.6 nmol/l in treated animals). However, the mean T4 concentration was found to be within the respective historical control range (29.73-54.92 nmol/l) and 3 of 10 treated animals marginally exceeded this range. This marginal finding, which did not correlate with other thyroid hormone levels, is not considered to be sufficient to identify a true thyroid modulator.

These data demonstrating a lack of endocrine-related effects impacts the need for a replication of the two-generation reproduction study reported by Schneider *et al*. (2005). NTP suggested that this study should be repeated as a Reproductive Assessment using Continuous Breeding (RACB) protocol citing enhanced sensitivity of the RACB for DEHP over a two-generation study as a rationale. However, the greater sensitivity is questionable given the findings of Schneider *et al*. For the RACB study of DEHP, the CERHR Expert Panel described the strengths and weaknesses as follows:

> "Clearly, a major strength of this study is the number of doses evaluated. The relatively small group sizes were compensated by the unusually high numbers of groups and the very low doses used. An additional strength is the fact that more offspring were evaluated early for alterations in the development of the reproductive system; a weakness might be that not all animals were so evaluated. The quality of the histology is another strength. The lack of vacuoles[3] is perplexing, but not lethal to the study."

In the Schneider *et al* study, all pups were examined for gross abnormalities; given the larger sample size used in this study design compared with the NTP RACB study of DEHP, the Schneider *et al* study is more robust for statistical comparisons. Therefore, repeating the study would not provide greater sensitivity to detect effects especially since the NOAEL was 450 mg/kg/d.

Taken together these additional data shed light on the uncertainty regarding endocrine disruption, and the two-generation study by Schneider *et al* confirm the absence of endocrine disruptive properties. We, therefore, do not see that further animal testing is justified.

- 2-Ethyhexyl *p*-**methoxycinnamate is not genotoxic and does not produce promote initiated cells.**

[3] This comment refers the vacuoles in the Sertoli cells of male rats exposed to DEHP for 13 weeks identified in a previous Expert Panel review as biologically significant.

Under the original NCI program, carcinogenicity studies of 2-ethyhexyl *p*-methoxycinnamate were recommended. The basis for the recommendation was the potential to hydrolyze to 2-ethylhexanol (2-EH) and cinnamic acid (CA), both suspected of being tumor initators. Much data have been generated on these two substances since that recommendation. 2EH has been shown to be a weak carcinogen in rodents (Astill *et al*, 1996) and not genotoxic. For cinnamates, recent regulatory reviews have not indicated any human health concerns. Furthermore, EHMC was investigated by industry in a series of valid regulatory toxicity studies, which have been evaluated and published as summaries by the former EU Scientific Committee on Cosmetology in 1996 (SCCP Opinion concerning 2-ethylhexyl-4-methoxycinnamate, 24 May 1996). All of them support the safety of the substance as only very low toxicity even after repeated sub chronic administration of high dosages was observed. In particular, only minor findings in clinical pathology or adaptive and physiological processes at high dose levels, such as liver weight or kidney weight increases, c ould be detected and no tissue changes were observed which might have indicated a potential oncogenicity after long-term exposure.

The skin tolerability of EHMC in rats, guinea pigs and man was found to be high even after repeated application of the undiluted substance. Generally the substance did not induce signs of irritation in the different test species. In addition, no signs for photoirritancy have been observed in humans. Therefore, an increased risk for skin cancer by an irritating or photoirritating effect is very unlikely. There is also no information available that EHMC might adversely change the protective properties of the epidermis (such as an influence on optical properties). Furthermore, more recent *in vitro* penetration experiments with human skin showed that the substance when applied in a sunscreen formulation remains almost entirely in the horny layer and is not likely to reach the living parts of the epidermis or the dermis (Potard et al[4].,)

The available photogenotoxicity studies do not indicate a photomutagenic effect by a toxic metabolite, free radicals or by degradation products. On the contrary, in a regulatory photocarcinogenicity study in hairless mice, EHMC was applied dermally at a 5% test concentration as a reference compound over a period of 40 weeks (A. Fourtanier, 1996[5]). There was no evidence of a photocarcinogenic effect, rather EHMC delayed the median latent period for tumor development by 2 weeks. So there is no indication that EHMC might be a tumor promoter on the skin.

[4] Potard et al., The Stripping Technique: In vitro Absorption and Penetration of Five UV Filters on Excised Fresh Human Skin, Skin Pharmacol Appl Physiol 13:336-344, 2000

[5] A. Fourtanier, Mexoryl SX protects against solar simulated UVR-induced photocarcinogenesis in hairless mice. Photochemistry and Photobiology, 64(4), 688-693, 1996

In summary, it can be stated that although no carcinogenic assay has been performed with EHMC to specifically test the carcinogenic potential, the available data clearly indicate that an increased risk of a carcinogenicity even following UV exposure is not to be expected. The favourable toxicological profile of EHMC (in particular no specific organ toxicity and no mutagenic or photomutagenic potential), make the induction or the enhancement of skin cancer very unlikely. In addition, the chemical structure of the substance does not give alert of a close relationship to known chemical or photochemical carcinogens. Therefore, a long-term carcinogenicity study for EHMC is considered not to be of high priority. On the contrary, it can rather be expected that the substance would provide protection against UV induced skin tumours in such an experiment.