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October 24, 2016

Michelle Ramirez
California Environmental Protection Agency
Office of Environmental Health Hazard Assessment
1001 I Street
Sacramento, CA 95814

Electronic submission to: P65Public.Comments@oehha.ca.gov

Subject: 2016 CIC Prioritization

Dear Ms. Ramirez:

FMC Corporation (FMC) is a developer, manufacturer, and distributor of products containing the pesticide active ingredient bifenthrin which are sold and used in California to control pests in agricultural crops as well as on lawns, gardens, sport fields, and golf courses. FMC is committed to working with California EPA to encourage practical, science-based regulation of our products.

FMC supports science based regulation, using a comprehensive risk assessment approach to ensure our products can be used without causing unreasonable harm to either human health or the environment. FMC appreciates the opportunity to provide comment on the California Office of Environmental Health Hazard Assessment's (OEHHA) posting dated September 9, 2016, of the Prioritization 2016: Chemicals for Consultation by the Carcinogen Identification Committee.

Specifically, FMC herein provides comments regarding bifenthrin which is listed in the chemical group Type I Pyrethroids background information document found on the OEHHA website. FMC believes bifenthrin should not be considered as a pure Type I pyrethroid. The OEHHA listed pyrethroids should each be considered separately due to the data being compound specific. Further, FMC believes bifenthrin should not be included for Prop 65 prioritization as it is not regulated as a carcinogen with a Q1* in the US and is not considered a carcinogen by WHO. Detailed comments are included in the following two attachments to this cover letter:

Attachment A: **FMC Corporation comments on Bifenthrin concerning OEHHA's Prop 65 Prioritization 2016: Chemicals for Consultation by the Carcinogen Identification Committee**

Attachment B: **Cohen, S., Expert opinion regarding the two-year bioassays in mice and rats for bifenthrin. 2011**

If you have any questions concerning these comments, please contact me by telephone at (215) 299-6717 or email at tim.formella@fmc.com.

Sincerely,



Timothy M. Formella

Senior Product Registration Manager

Attachment A

FMC Corporation comments on Bifenthrin concerning OEHHA's Prop 65 Prioritization 2016: Chemicals for Consultation by the Carcinogen Identification Committee

FMC Corporation comments on Bifenthrin concerning OEHHA's Prop 65 Prioritization 2016: Chemicals for Consultation by the Carcinogen Identification Committee

Bifenthrin is a pyrethroid insecticide that is registered worldwide and is not regulated as a carcinogen (Q1*) by the US EPA. Its chemical structure and mechanism of toxicity is not clearly a Type I or Type II pyrethroid; it should be considered as a mixed type pyrethroid and should not be evaluated together with the class of Type I pyrethroids. Furthermore, 'Type I' refers to the impact and biochemical binding on the nerve synapse, whereas the carcinogenicity assessment is on a completely different biochemical effect involving secondary exposure tissues. Therefore, a case-by-case assessment for carcinogenicity based upon the AI's specific pharmacodynamic and ADME properties is more appropriate than combining pyrethroids together in one assessment.

Mode of action class: Bifenthrin is not a Type I pyrethroid

Bifenthrin is sometimes erroneously referred to as a Type I pyrethroid based upon its chemical structure because it lacks a α -cyano group in the alcohol moiety, similar to a Type II pyrethroid; however, it has an ortho-methyl biphenyl alcohol in place of the more traditional 3-phenoxybenzyl moiety. Molecular modeling of the bifenthrin alcohol moiety versus Type I and II alcohols indicates that the o-methyl group can occupy a similar space to the α -cyano group of cypermethrin, which may be the basis for some Type II pyrethroid activity. In addition, its neurophysiological mechanism is not consistent with a Type I pyrethroid. In the intact electrode-implanted cockroach nerve assay, bifenthrin fails to cause prolonged repetitive firing in sensory nerves, an effect typical of Type I pyrethroids. An in vivo assay commonly used to characterize Type I versus Type II pyrethroids is the Acoustic Startle Response (ASR) in the rat. Type I pyrethroids cause an increase in ASR whereas Type II cause a decreased ASR. In a developmental neurotoxicity study, bifenthrin reduced the ASR in female pups at PND20, but the effect reversed at PND60. In human nerve voltage-gated sodium channel experiments, bifenthrin showed features of both Type I and Type II pyrethroids. These data

demonstrate that bifenthrin is not a classical Type I pyrethroid and should be considered as a mixed Type I and Type II pyrethroid.

Global carcinogenicity evaluations

Bifenthrin is not regulated as a carcinogen globally. It is not considered a carcinogen by the World Health Organization (WHO) or the FAO/WHO Joint Meeting on Pesticide Residues (JMPR). According to the WHO Specification and Evaluations for Public Health Pesticides summary of January 2012: "Bifenthrin has been evaluated by the WHO IPCS (International Programme on Chemical Safety) (2000-2002, Report No. WHO/PCS/1.50) and by the FAO/WHO JMPR in 1992 and 2009. The JMPR concluded that the results of the long term studies in rats and mice and a series of studies designed to evaluate genotoxicity indicated that bifenthrin is unlikely to pose a carcinogenic hazard to humans."

In the US, the bifenthrin toxicology database is complete with toxicological evaluations done for all required studies for registration. The US EPA does not have concerns about bifenthrin as a carcinogen (no Q1* assigned) and does not regulate it as such.

According to US EPA (Federal Register notice of September 14, 2012; document number 2012-22772): "Bifenthrin is classified as a 'possible human carcinogen' based on an increased incidence of urinary bladder tumors in mice." However, the EPA concluded that "the bladder tumors may not be uncommon in mice and are not likely to be malignant. Additionally, these tumors were observed only in male mice at the highest dose tested and the incidence was of borderline significance. No evidence of carcinogenicity was observed in bifenthrin carcinogenicity studies in rats, and bifenthrin was negative in five different tests for mutagenicity." Because of this, EPA recommended a non-linear approach and no Q1* was derived. This recommendation of not assigning Q1* indicates that the tumorigenic potency of bifenthrin is very minimal and further demonstrated that there is no major concern for carcinogenicity of bifenthrin.

In the EU, bifenthrin is not considered a known or even a probable carcinogen. It is considered a possible carcinogen based on borderline evidence by ECHA (H351; IARC

Carc. Category 3). This is the lowest level of classification possible for a pesticide active ingredient. This classification has not limited or restricted any uses.

This classification in the EU is based on slightly increased urinary bladder lesions/tumors in males at the high dose of 600 ppm observed in the same mouse oncogenicity study noted by the US. No similar lesions were seen in the female mice. The high dose in mice exceeded the MTD (maximum tolerated dose) and caused excessive animal deaths and ~20% reduction in body weight gain during the first 28 and 90 days of the study. Additionally, the high dose far exceeds the anticipated exposure level that humans would experience from typical uses of bifenthrin.

Weight of evidence

Following a scientific weight-of-evidence approach, these additional points should be considered that clearly show bifenthrin should not be classified as carcinogenic:

- Bifenthrin does not cause genotoxicity (non-genotoxic).
- There is no evidence of treatment-related tumors in rats.
- There are no urinary bladder tumors in female mice in the 18-month oncogenicity study.
- There is not a statistically significant ($p < 0.05$) pairwise increased incidence of bladder lesions/tumors at dose levels up to 500 ppm (MTD) in male mice.
- It is well accepted among the scientific community and regulatory bodies that the type of urinary bladder lesions seen in the high-dose males are unique to the mouse, are not carcinoma-type tumors, do not metastasize, are not invasive, and can generally arise from inflammatory or disruptive disorders.
- The lymphoid tumors (noted in female mice) are not considered to be treatment-related and were not statistically significantly different than historical controls.
- The lung tumors (noted in female mice) are not considered to be treatment-related and were not statistically significantly different than historical controls.
- The high dose (600 ppm) at which effects were seen in the mouse carcinogenicity study far exceeded the MTD. If the highest dose of 600 ppm is excluded from the analysis due to exceedance of the MTD, which is typical for

carcinogenicity studies, then there would be a complete absence of data supporting classification of bifenthrin as a carcinogen.

A detailed response to the OEHHA comments on relevant studies is provided below:

OEHHA description:

• *Long-term feeding studies in mice*

- *20- to 21-month studies in male and female Swiss mice: Geiger (1986), as reviewed in ECHA (2009)*
- *Increase in urinary bladder tumors (by pairwise comparison) in males*
- *Increases in lymphoblastic leukemia and lung tumors (by pairwise comparisons) in females*

FMC response:

In the Geiger (1986, A83-974) study, Swiss mice (50 animals/sex/group) were treated with bifenthrin for 20 to 21 months at doses of 0, 50, 200, 500, or 600 ppm via dietary administration. A re-evaluation of the histological slides was conducted for 1) urinary bladders of all males and females, 2) liver sections of all male mice, and 3) lung sections of all female mice. All slides were reviewed in a blind evaluation by a peer-review pathologist (Butler 1991). Slides with urinary bladder lesions were reviewed by two additional pathologists (Butler et al., 1997). Statistical analysis of the urinary bladder findings was based on the majority opinion of the panel of 3 expert pathologists. A summary of the urinary bladder lesions/"tumors" and lung tumour findings (original evaluation and re-evaluation) is presented below:

Urinary bladder tumors/lesions male mice

Table 1 contains both the original urinary bladder tumor histopathological classification data together with the re-evaluated tumors/lesions histopathology data (Butler 1991; Butler et al., 1997).

Table 1: Incidence of tumors/lesions in the urinary bladder in male Swiss Webster mice

Tumour type	Ctr	50 ppm	200 ppm	500 ppm	600 ppm	Trend test	Reference
Leiomyosarcomas	4% (2/48)	12% (6/50)	16% (8/50)	14% (07/50)	29%** (14/49) p<0.01	positive with p=0.0005	Geiger 1986
Submucosal mesenchymal urinary bladder tumours	12	14	16	16	27 p=0.07	positive p=0.046	Butler 1991 Butler et al., 1997
Submucosal mesenchymal urinary bladder tumours including early lesions	14	14	18	16	30 p=0.05	positive p=0.033	Butler 1991 Butler et al., 1997

The bladder lesions/"tumors" were examined by a world-renowned bladder pathologist team, who concluded that the tumors were not carcinogenic due to the lack of any evidence of metastases, and these tumors are not relevant to humans. In Butler (1991), the tumors originally described as leiomyosarcomas were re-diagnosed as non-invasive submucosal mesenchymal tumors. Butler et al. (1997) reconfirmed that those are really non-invasive submucosal mesenchymal tumors or lesions, and the statistical significance should be determined using the pattern/incidence of submucosal mesenchymal lesions.

Dr. Samuel Cohen of the University of Nebraska Medical Center evaluated these studies and provided his expert opinion as well (2011). He corroborated that the tumors were of submucosal mesenchymal origin and stated his overall histopathological interpretation that the mesenchymal lesions in the mouse urinary bladder presented benign proliferations in the mouse urinary bladder, but did not metastasize. The tumors are described to occur predominantly in the submucosa occasionally extending into the

muscle layer. According to Cohen, this does not represent muscle invasion, as they did not destroy the muscle layers themselves. Cohen stated “Whether these lesions actually represent benign neoplasms or whether they represent an aberrant inflammatory and granulation tissue response continues to be debated, although the evidence increasingly suggests that it is an inflammatory, reactive disorder”.

Marginal increases in urinary bladder lesions/tumors were observed in male mice only at the high dose that clearly exceeded the maximum tolerated dose (MTD). Additionally, the increases were not statistically significant ($p < 0.01$) (Butler 1991, Butler et al., 1997). All of the mice at the high dose of 600 ppm exhibited clinical signs (tremors) as well as 18% decrease in mean body weight gain at 90 days; 26% decrease in body weight gain at 28 days; and 2/50 females and 2/50 males died in the 2nd week. In the range-finding 28-day mice study, 1000 ppm caused 10/10 females death by day 12 and 7/10 male death by day 7; 600 ppm caused 20% (2/10) animal death in female mice (Rand 1983). If the highest dose of 600 ppm is excluded from the analysis due to exceedence of the MTD, which is typical for carcinogenicity studies, then there would be a complete absence of data supporting classification of bifenthrin as a carcinogen.

Lymphoid tumors in female mice

The incidence of lymphoid type tumors in the female mice is not considered treatment-related due to the lack of a clear linear dose relationship and the lack of statistical significance ($p < 0.01$). Additionally due to the physiology of the lymphoid system, the incidence of lymphoid leukemia and other lymphoid tumors should be assessed together. (Combining of these types of lymphoid tumors is common histopathology practice). Table 2 contains both the original lymphoblastic leukemia data together with the combined lymphoid tumors (including lymphoblastic leukemia) data as summarized in the EU Draft Assessment Report (2006).

Table 2: Lymphoid tumors in female mice

Tumour type	Control	50 ppm	200 ppm	500 ppm	600 ppm	Reference
Lymphoblastic leukemia	24%	28%	34%	20%	44%* p=0.02	Geiger 1986
Lymphoid tumors (including lymphoblastic leukemia)	38%	38%	40%	32%	47%	EU bifenthrin Draft Assessment Report 2006

For lymphoblastic leukemia alone, a large number of control animals were affected (24%). Although the incidence in the high dose females was considered statistically significant ($p=0.02$) in the original report, it was not $p<0.1$ and the trend test did not show statistical significance. In addition, the dose response is not monotonic (20% incidence at 500 ppm is less than the incidence in controls).

When combining all types of lymphoid tumors, including lymphoblastic leukemia, there was no statistical significance in pairwise comparisons. More control animals were affected (38%) than in some of the treated groups. The dose response was not monotonic (incidence of 32% at 500 ppm is below the incidence in controls). The study pathologist concluded that the observed incidence pattern was not treatment-related. Additionally, the European Chemicals Agency (ECHA) did not consider the lymphoid tumors as treatment-related in their evaluation (ECHA 2011).

Lung tumors in female Swiss mice

The incidence of lung tumors in the female mice is not considered dose-related due the lack of a clear linear dose relationship, a lack of statistical significance ($p<0.01$) and a negative trend test (Table 3). In addition, Butler (1991) re-evaluated the histological slides of the female mice lungs and concluded that many tissues were misidentified as

adenocarcinomas when they were actually benign adenomas (non-malignant, non-invasive tumors).

Table 3: Lung tumors in female mice

Tumor type	Control	50 ppm	200 ppm	500 ppm	600 ppm	Trend test	Reference
Bronchio-alveolar adenomas	0	2%	0	6%	2%	negative	Geiger 1986
Bronchio-alveolar adenocarcinomas	28%	50%	46%	32%	46%	negative	Geiger 1986
Bronchio-alveolar adenomas and carcinomas	28% (14/50)	52%* (26/50) p=0.012	46%* (23/50) p=0.048	38% (19/50)	48%* (23/48) p=0.041	negative	Geiger 1986
Bronchio-alveolar adenomas	24%	44%* p=0.029	38%	30%	40%	negative	Butler 1991
Bronchio-alveolar adenocarcinomas	4%	8%	8%	8%	4%	negative	Butler 1991
Bronchio-alveolar adenomas and carcinomas	28%	52%* p=0.013	46%* p=0.049	38%	44%	negative	Butler 1991

Butler (1991) stated “*The study pathologist used different criteria and considered the great majority of the neoplasms to be malignant on the basis of peripheral infiltration into the air space.*” Historically, as the study pathologist remarks, ‘*such neoplasms are primarily regarded as benign*’. I have utilized the conventional historical criteria and in the absence of direct evidence of malignant behaviour classify the lesions as adenoma (benign)”.

There is little difference between the two histopathological assessments of the combined lung tumors by Geiger (1986) and Butler (1991). The only difference refers to the incidences in the 600 ppm dose group (48% versus 44 % in the re-evaluation). The incidence of bronchio-alveolar adenomas and carcinomas were increased compared to concurrent controls (P values between 0.013 and 0.049), but there was already a relatively high incidence in the controls (28%) and the trend test was negative. In all treatment groups, there were tumor incidences of 38 to 52% compared to controls (28%), but there was no dose-response relationship. The conclusion by both the study pathologist (Geiger 1986) and the EU expert toxicologist reviewer as noted in the Draft Assessment Report of 2006 was that this incidence or pattern of lung tumors should not be considered treatment -related. ECHA also recognised that the mechanism of toxicity and pharmacodynamic properties of bifenthrin do not support a conclusion that the increased incidences of lung tumors in females is dose-related.

OEHHA description:

- *Long-term feeding studies in rats*
 - *104-week studies in male Sprague-Dawley and female Tac (SD) fBR rats: McCarty (1986), as reviewed in ECHA (2009)*
 - *No treatment-related tumor findings*

FMC response:

Bifenthrin did not show any potential to cause tumors when rats were fed concentrations of 0, 12, 50, 100, or 200 ppm of technical bifenthrin for 2 years. However, at 200 ppm there was toxicity demonstrating the MTD was reached.

OEHHA description - Genotoxicity:

- *As reviewed in US EPA (1992)*
 - *Salmonella reverse mutation assays, strains TA98, TA100, TA1535, TA1537, and TA1538 (+/-S9) (negative)*
 - *Hamster hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT) mutation assays in mouse lymphoma cells (negative)*

- *Forward mutation at the TK locus in mouse lymphoma cells (negative)*
- *Chromosomal aberration (CA) assays in Chinese hamster ovary (CHO) cells and rat bone marrow cells in vitro (negative)*
- *Unscheduled DNA synthesis (UDS) in rat hepatocytes in vitro (negative)*
- *Sister chromatid exchange (SCE) in CHO cells in vitro (negative)*

- *As reviewed by ECHA (2009)*
 - *CA in rats in vivo (negative)*
 - *Micronucleus (MN) formation in mouse bone marrow in vivo (negative)*
 - *UDS in rat hepatocytes in vivo (negative)*

FMC response

FMC agrees with both the USEPA and ECHA evaluation and conclusion that bifenthrin does not cause genotoxicity (non-genotoxic) based on a full battery of *in vitro* and *in vivo* genotoxicity studies. Therefore, these assays indicate that bifenthrin is not a carcinogen through a genotoxic mechanism.

References

Rand GM (1983). Twenty Eight day range finding study in mice with FMC 54800 technical. FMC study number A83-839/A83-839A.

ECHA (2011). Committee for Risk Assessment RAC: Opinion proposing harmonised classification and labelling at Community level of bifenthrin.

EU Draft Assessment Report: Bifenthrin (2006).

Butler WH (1991). FMC 54800 technical oncogenicity lifetime feeding study in Albino mice histopathological review of selected sections of liver, lung and urinary bladder.

Butler WH, Cohen SH, Squire RA (1997). Mesenchymal tumors of the mouse urinary bladder with vascular and smooth muscle differentiation. *Toxicol Pathol.* 1997, 25(3):268-74.

Geiger LE, Barbera J and Ballester EJ (1986). Oncogenicity study of FMC 54800: lifetime feeding study in Albino mice. FMC number A83-974.

FMC Corporation
October 24, 2016

Attachment B

**Expert Opinion Regarding the Two-Year Bioassays in Mice and Rats
for Bifenthrin; Cohen 2011**

**EXPERT OPINION REGARDING THE TWO-YEAR
BIOASSAYS IN MICE AND RATS FOR BIFENTHRIN**

A handwritten signature in black ink, appearing to read 'S. M. Cohen', is centered on the page.

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March 28, 2011

SUMMARY

Bifenthrin induced a lesion in male mouse bladder which is classified as mesenchymal proliferative lesion, which is a benign proliferation mainly in the submucosa. It is specific to the mouse, not occurring in other laboratory test species, not in veterinary or zoological animals, and not in humans. In addition, there was an equivocal increased incidence of liver tumors in male mice. If actually related to bifenthrin treatment, like with other pyrethroids liver tumors occur by a mode of action that is similar to Phenobarbital involving binding to the CAR receptor, induction of certain P450 isozymes, and increased hepatocellular proliferation. This mode of action has been demonstrated to be rodent specific and not relevant to humans. The Risk Assessment Committee concluded that bifenthrin is nongenotoxic in vivo, that the lung tumors and lymphomas in female mice were unrelated to treatment and that the rat bioassay was negative. Based on those conclusions and the lack of human relevance of the male mouse bladder and liver tumors, it can be confidently concluded that bifenthrin does not pose a cancer hazard to humans and should not be classified as a carcinogen.

INTRODUCTION

I have read the Committee for Risk Assessment (RAC) document regarding bifenthrin dated February 15, 2011. I have been asked by FMC to respond to issues raised by RAC regarding classification of the carcinogenicity of bifenthrin.

I first became involved with an evaluation of the two-year bioassay of bifenthrin in 1989 based on the development of urinary bladder lesions in the mouse bioassay. This led to a more extensive review of the pathology slides by Dr. William Butler and Dr. Robert Squire. Based on their findings and on my previous experience with this unusual lesion, we ultimately published a manuscript describing the findings. Those findings are referred to in the RAC document as the Butler review of the pathology.

RAC CONCLUSIONS

As indicated in the RAC document, they have concluded that the two-year bioassay in rats was negative for tumorigenicity, an opinion for which I concur. In mice, they indicated that there was an increased incidence of lung tumors and lymphomas in the female mice, but that for a variety of reasons which they describe, they concluded that these were not treatment related nor did they indicate a carcinogenic risk for humans. This conclusion regarding lymphomas was based on the very high incidence in controls, lack of statistical significance when all lymphomas are combined (a common practice in evaluating rodent bioassays), and lack of a dose response. In addition, there is considerable doubt as to the human relevance of these lymphomas in mice because of their association with endogenous mouse retroviruses that are present in nearly all mice, experimental as well as the wild. The conclusion regarding the female mouse lung tumors was based on the very high incidence in controls and the lack of a dose response. I concur with the conclusions regarding lymphomas and lung tumors. In contrast, the RAC concluded that there were significant issues regarding a possible carcinogenic hazard for humans based on the findings of increased bladder tumors in male mice and a slight, but not statistically significant,

increase in liver tumors in male mice. My opinion will be devoted to a discussion of these two tumor findings in male mice.

The RAC also concluded that bifenthrin is not an *in vivo* genotoxic agent. I concur with this conclusion, based on negative findings in *in vivo* cytogenetics, micronucleus, and unscheduled DNA synthesis assays. Most of the *in vitro* assays, including the Ames assay, chromosomal aberrations, unscheduled DNA synthesis in hepatocytes, and sister chromatid exchange, were all negative. The only positive finding *in vitro* was the mouse lymphoma mutagenicity assay, but this was positive only at extremely high concentrations which likely were cytotoxic. Supporting such a conclusion was equivocal to negative findings in the HPRT assay, which was only marginally positive at cytotoxic concentrations. The conclusion that bifenthrin will not be a mutagen or genotoxic agent *in vivo* is strongly supported by the data. This is also similar to the conclusion that has been reached for pyrethrins and pyrethroids, in general, the class of chemicals to which bifenthrin belongs. Based on the conclusion of non-genotoxicity, any carcinogenic effect in rodents would be by an indirect, non-genotoxic mechanism.

The RAC raised concerns about the male mouse liver and bladder lesions, but clearly they had difficulties in reaching a conclusion as to whether this posed a true cancer hazard or risk for humans or not. As I will present in a discussion of each of these tumors, I believe that the data clearly show that they do not pose a hazard to humans, and bifenthrin does not pose a cancer risk for humans for any tissues.

LIVER TUMORS

Incidences of the liver tumors in the mouse were slightly increased, but there was not a clear statistical significance between the various dose groups and the controls. There was considerable discussion by the RAC concerning the statistical significance of these lesions and whether they are biologically related to the treatment. I do not believe that is the key issue on which to decide the relevance of these liver findings in male mice for humans. Pyrethroids frequently have shown evidence of inducing liver tumors in rats and/or mice (Yamada et al., 2009). When this has occurred, it has clearly been shown that it is due to a mode of action involving interaction with the constitutive androstane receptor (CAR) which leads to induction of various cytochrome P450 enzymes, leading to an increase in hepatocellular proliferation and ultimately the induction of liver tumors. This is the same mode of action that has been demonstrated for phenobarbital, an anti-epileptic drug that has been used in humans for several decades (Whysner et al., 1996; Ross et al., 2010). The effect of the various pyrethroids on cytochrome P450 induction is considerably less than seen with phenobarbital, and related to this, the tumor induction effects have also been less (Yamada et al., 2009). In some instances, such as for bifenthrin, the liver tumor induction effect is equivocal. The significance of this relationship is that this mode of action is clearly not relevant to humans. For phenobarbital, it has been demonstrated that mice that have had the human receptor inserted in place of the mouse receptor for CAR, there is evidence of the induction of cytochrome P450 and effects on metabolism, but they do not show the proliferative effects on the hepatocytes seen with the mouse receptor (Ross et al., 2010). In addition, there have been extensive epidemiology studies of patients who have taken phenobarbital for several years to demonstrate that it is not a carcinogen in humans (Whysner et al., 1996). This negative finding regarding cancer risk in humans with phenobarbital is at dosages in humans that

approach those that were actually used in the mouse and rat studies that showed a tumorigenic effect. This similarity in dose for phenobarbital between humans and rodents is in marked contrast to the drastic differences in exposures to pyrethroids in the rodent studies compared to human exposures. Thus, the liver tumors in male mice with bifenthrin, if treatment related, occur by a mode of action that is not relevant to humans. Therefore, either the tumors were not treatment related or they were induced by a mode of action not relevant to humans. Either way, they represent no hazard for human cancer development.

URINARY BLADDER LESIONS

The mouse bladder lesions are a bit more confusing, primarily because of their unusual histopathological appearance and uniqueness to the mouse. These lesions were originally diagnosed by the study pathologist as leiomyomas and/or leiomyosarcomas. In the review by myself and by Drs. Robert Squire and William Butler, we concluded that these actually are a lesion which has had a variety of names appended to it (Butler et al., 1997). The review by Dr. Butler clearly demonstrated that they were more common in all groups, including the controls, than had originally been observed by the study pathologist, in part, because of the small size of several of the lesions. A preferred term for these mouse bladder lesions has been recommended to be mesenchymal proliferative lesion (see below).

These mesenchymal proliferative lesions of the mouse bladder have been observed only in a few strains, primarily those that were derived from the Swiss mouse, including the CD-1 mouse strain used in the studies with bifenthrin (Halliwell, 1998). The morphology is quite unusual, but

gives some clues as to the possible pathogenesis and biological nature of the lesions. These tumors can range in size from microscopic (< 1 mm.) to grossly visible, several millimeters in greatest dimension. They occur predominantly in the submucosa, but occasionally extend into the muscle layer. However, this does not actually represent muscle invasion, as it does not destroy the muscle layers themselves, but grows in the connective tissue that is between the various muscle bundles in the bladder wall. They do not extend beyond the serosa and they never metastasize. An indication of their overall benign biological nature is the fact that they never have been observed to penetrate the overlying epithelial layer, despite being in close proximity to it.

By light microscopy, most of these lesions consist of two cell types (Jacobs et al., 1976; Butler et al., 1997; Halliwell, 1998). One cell type is epithelioid in nature, with eosinophilic cytoplasm and pleomorphic nuclei, frequently with sharp angulations. The nuclei tend to be centrally located and can contain eosinophilic or basophilic inclusions. The spindle cells tend to be small, with oval nuclei, and usually distinctly separate from the epithelioid cells. At the periphery of these lesions, there was frequently a chronic inflammatory infiltrate consisting of lymphocytes, histiocytes, and occasional plasma cells. Some lesions showed an acute inflammatory infiltrate of polymorphonuclear leukocytes. Also seen in some of these lesions, particularly the larger ones, were foreign body giant cells. Intermixed with the tumor cells was hemosiderin deposition. Occasional mitoses were seen in some lesions, particularly the larger ones, along with necrosis.

By electron microscopy, no evidence of the types of cell junctions that would be indicative of epithelial cells were detected. Furthermore, a few of these tumors have been stained

immunohistochemically for keratin, a marker for epithelial cells, and have been negative. In contrast, smooth muscle differentiation was present, including myofilament bundles. However, by histology and by transmission electron microscopy, there was also evidence of fibroblastic differentiation. The cell type of origin most likely is the pericyte, which can show evidence of smooth muscle differentiation, but the cell of origin could also represent a myofibroblast. The lesions tend to be centered around small blood vessels, which may explain the presence of hemosiderin in these lesions.

When the epithelioid cell type predominates, the lesions have frequently been misdiagnosed as invasive carcinomas. However, there is never a connection of these lesions to the overlying epithelium, and there is no evidence histologically, immunohistochemically, or electron microscopically of epithelial differentiation. The lesions are clearly composed of mesenchymal cells. Because of the presence of some smooth muscle features, they were called smooth muscle tumors. They did not have the long spindle cells with cigar-shaped nuclei characteristic of leiomyomas or well differentiated leiomyosarcomas, and they did not have the marked pleomorphism and high mitotic rate of high grade leiomyosarcomas.

The atypia that is present in many of these lesions, particularly if epithelioid cells dominate, are suggestive of possible malignancy. However, it should be kept in mind that mesenchymal lesions frequently can show a considerable degree of atypia even if they are benign tumors or reversible inflammatory lesions (Weedon, 2002). In human pathology, an example of a benign tumor with considerable cellular atypia is the atypical fibroxanthoma, which occurs in the dermis. A typical example of an inflammatory lesion with considerable atypia is nodular fasciitis.

The presence of the mesenchymal lesions in the smooth muscle wall of the bladder in a couple of instances suggested the possibility of invasion. However, these lesions are not actually in the muscle fibers, but in the connective tissue that is intertwined between the muscle fibers of the detrusor muscle of the urinary bladder. A clear indication of a lack of invasion capability is the uniform finding that these lesions never invade the overlying epithelium even though they approach the basement membrane. Thus, the overall interpretation of these mesenchymal lesions is that they represent benign proliferations in the mouse urinary bladder.

Whether these lesions actually represent benign neoplasms or whether they represent an aberrant inflammatory and granulation tissue response continues to be debated, although the evidence increasingly suggests that it is an inflammatory, reactive disorder.

HUMAN RELEVANCE OF URINARY BLADDER LESIONS

These lesions were originally described in the 1950s by the group in England that were studying bladder cancer in mice by implanting pellets of various composition directly into the mouse bladder (Bonser and Jull, 1956; Roe, 1964). They were observed around some of the sutures in the bladder wall following the surgical implantation and were referred to as “vegetative bodies”. Although they were originally thought to be epithelial in nature, they were clearly considered to be inflammatory and a reaction to the presence of the suture and/or a reaction to the surgical procedure itself. It was not until our publication in 1976 (Jacobs et al., 1976) that the mesenchymal nature of these lesions was clearly demonstrated. In subsequent examples

following administration of various chemicals without surgical manipulation of the bladder, the lesions almost always occurred in the trigone region of the bladder, when location could actually be ascertained. In several of the examples, there was associated blockage of the ureter and associated hydroureter and hydronephrosis, possibly indicating, again, an inflammatory process.

In the 1990s, a committee was organized under the auspices of the International Life Sciences Institute (ILSI), including representatives from the Food and Drug Administration and Environmental Protection Agency, to analyze examples of this lesion that had been observed with a variety of pharmaceutical drugs in preclinical development. This group of individuals reviewed a large number of examples (approximately 100), and was reported by Halliwell (1998). I was a member of that panel. The group concluded that the lesions were clearly benign, and most likely represented an inflammatory process, as there was always a chronic inflammatory infiltrate and hemosiderin deposition present in these lesions.

These lesions have been occasionally described in association with several pharmaceutical agents, and in no case has it been considered to be relevant as a cancer risk in humans, so this finding has never been included in the package insert information for these pharmaceutical agents, either in the USA or in Europe.

Origin from blood vessels, particularly the possibility of pericytes, was considered most likely, given the presence of hemosiderin in most of the lesions, including those which were quite small.

Over the past two decades, this lesion has increasingly been identified in two-year bioassays in mice, with the incidence higher in male mice than in female mice. The frequency with which they have been identified may well be related to the greater awareness of toxicologic pathologists of this lesion. However, the lesion still is occasionally misdiagnosed as invasive carcinoma, particularly if it is predominantly epithelioid in appearance.

Similar lesions have also been identified in the kidney pelvis (referred to in Butler et al., 1997), which is actually an extension of the urinary bladder, so it is not surprising that such lesions might occur there, as well. However, in addition to the urinary tract, I have seen a couple of examples of this in the seminal vesicles, which also were described by Halliwell (1998). Karbe (1999) demonstrated that the lesions seen in the urinary bladder are identical to what has been referred to as deciduoma in the female mouse uterus, although that term may actually be misleading. It was referred to as deciduoma because of the decidual-like appearance of the cells, primarily large cells with central nuclei and significant amounts of eosinophilic cytoplasm, so called decidualization of the endometrial stroma. It may well be that the lesion in the endometrium is the most common site for this particular lesion.

Based on our increasing understanding of the biology of this particular lesion in the urinary bladder, the recommended terminology is “mesenchymal proliferative lesion” as recommended by the INHAND Project (International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice), which is a joint initiative of the Society of Toxicologic Pathology (STP), European Society of Toxicologic Pathology (ESTP), the British Society of Toxicologic Pathology (BSTP) and the Japanese Society of Toxicologic Pathology (JSTP).

HUMAN PATHOLOGY

It is clearly benign, and is most likely inflammatory in nature. However, to better understand its relevance to human risk, one has to realize that it has never been described in any tissue of any other species. It has not been observed in the urinary bladder or any other tissue of rats, hamster, monkeys, or dogs in toxicologic studies (Cohen, 1983), and it has not been reported in the veterinary pathology literature of autopsies on a large number of domesticated and zoo animals (Pamukcu, 1983). Most importantly, it is never been reported in humans (Koss, 1975; Murphy et al., 1994; 2004; Eble et al., 2004).

In addition to having been involved with research on bladder carcinogenesis since 1964, I am also a board certified pathologist for human pathology (board certified in 1976). I have specialized in surgical pathology with an emphasis on urologic pathology, and I have never seen any lesion that is the same as this mesenchymal proliferative lesion seen in the mouse bladder, seminal vesicle or endometrium. Furthermore, no such lesion has ever been described in the world's literature for the urinary bladder or any other tissue. I have also shown slides of this lesion to internationally recognized expert pathologists that have spent their careers investigating the urinary bladder, including Drs. Leopold Koss (Sloan Kettering and Montefiorie Hospital, New York City), George Farrow (Mayo Clinic) and William Murphy (University of Tennessee and the University of Miami). These individuals have been authors of the Armed Forces Institute of Pathology fascicles on urinary bladder, Dr. Koss having written the fascicle for the second series (Koss, 1975), Drs. Murphy and Farrow being authors of the third series (Murphy et al.,

1994), and Dr. Murphy being one of the authors for the current, fourth series (Murphy et al., 2004). They have also indicated that they have not seen such a lesion in humans. Furthermore, this lesion was not mentioned in the most recent WHO monograph on bladder lesions (Eble et al., 2004).

Literally tens of thousands if not hundreds of thousands of biopsies of the bladder and millions of curettage specimens of the endometrium of women of all ages are examined world-wide each year. Until the past decade or so, seminal vesicles were uncommonly examined in surgical pathology, but with the increasing numbers of prostatectomies for prostate cancer, part of which includes histopathologic evaluation of the seminal vesicles for extra-prostatic spread, tens of thousands seminal vesicles are examined yearly. The fact that a lesion similar to the mouse mesenchymal proliferative lesion has not been reported in humans is clearly indicative that it does not occur in humans. Mesenchymal proliferative lesions of the mouse bladder have not been described in human bladder, endometrium or seminal vesicle nor in any other tissues in humans.

CONCLUSIONS

In summary, the bladder lesion that was associated with bifenthrin administration to male mice is a benign proliferation, most likely related to an inflammatory response, and unique to the mouse. Thus, it has no relevance to human cancer risk.

Based on the above discussion, it is clear that the tumors associated with bifenthrin administration in mice do not represent a human cancer risk, and therefore bifenthrin should not be classified as a carcinogen.

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