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

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

Date: September 12, 2019

SUBJECT: **Ethoxyquin:** New and updated DERs for guideline and non-guideline toxicity studies

PC Code: 055501
Decision No.: 549716, 549717, 549718
Petition No.: N/A
Risk Assessment Type: N/A
TXR No.: 0057939
MRID No.: See Table 1

DP Barcode: D450582, D450585, D453267
Registration No.: N/A
Regulatory Action: N/A
Case No.: N/A
CAS No.: 91-53-2
40 CFR: §180.178

FROM: Austin Wray, Ph.D., Toxicologist
Risk Assessment Branch IV,
Health Effects Division (7509P)

THROUGH: Kristin Rickard, Acting Branch Chief
Risk Assessment Branch IV
Health Effects Division (7509P)

TO: Brian Van Deusen, Risk Assessor
Risk Assessment Branch IV,
Health Effects Division (7509P)

I. ACTION REQUESTED:

The ethoxyquin human health risk assessor requested the lead toxicologist update existing data evaluation records (DER) from the toxicity database and compose DERs for new toxicity studies to support the ethoxyquin registration review.

II. BACKGROUND:

A number of DERs from the ethoxyquin toxicity database require revisions to conform to current HED policies and practices. It was determined during registration review that only those DERs having a direct impact on the human health risk assessment (i.e. those that were the basis for or impacted selection of a point of departure) were to be updated. The 90-day oral study in dogs (MRID 44148901) was the only existing DER that was updated to support registration review. In addition, new DERs were composed for guideline subchronic rat inhalation (MRID 50776901) and *in vitro*/*in vivo* dermal penetration studies (MRID 50776902) submitted in response to a data call-in (GDCI-055501-1542), and a non-guideline acute oral study in dogs (MRID 46336401) that was found in the database but was not reviewed previously.

III. RESULTS/DISCUSSION:

The title and MRID number of guideline and non-guideline studies that required new or updated DERs for the ethoxyquin registration review are listed in Table 1 below. Refer to the attached DERs for more information on those studies.

Table 1. Citation and MRIDs for guideline/non-guideline studies that required new or updated DERs for the ethoxyquin registration review.

Citation	MRID
Naas DJ. 1996. A 90-Day Oral (Capsule) Toxicity Study of Ethoxyquin in Dogs. WIL Research Laboratories, Inc. Ashland, OH. Study No. WIL-273013, October 24, 1996. Unpublished.	44148901
Harriman J. 2004. An acute reference dose oral (capsule) toxicity study of ethoxyquin in dogs. WIL Research Laboratories, Inc. Ashland, OH. Study Number WIL-273015. July 28, 2004. Unpublished.	46336401
Brooker A. 2019. Ethoxyquin: toxicity study by inhalation administration to Sprague Dawley rats for 4 weeks followed by a 2-week recovery period. Envigo CRS Limited, Alconbury, Huntingdon, Cambridgeshire, UK. Laboratory Study Number: TG86BD, January 7, 2019. Unpublished.	50776901
Jones A. 2019. Ethoxyquin: *In vitro* (human/rat) and *in vivo* (rat) dermal absorption. Envigo CRS Ltd., Cambridgeshire, UK. Study No.: BP05LT, January 24, 2019. Unpublished.	50776902

IV. CONCLUSIONS:

The DER for the subchronic oral toxicity study in dogs (MRID 44148901) was updated after re-evaluation of the data presented in the study report. Previously, the no-observed-effect-level (NOEL) and lowest-observed-effect-level (LOEL) were set at 2 mg/kg/day and 4 mg/kg/day,

respectively, based on elevated liver enzymes and microscopic findings in the liver (cytoplasmic vacuolation and hepatocellular necrosis). As part of this update, the adversity of these and other effects reported in the study were re-evaluated in order to establish a no-observed-adverse-effect-level (NOAEL) and lowest-observed-adverse-effect-level (LOAEL) for the study. It was determined that the effects in the liver including elevated enzyme levels, dark livers, and microscopic findings (bile duct hyperplasia, cytoplasmic vacuolation, and hepatocellular necrosis) were reflective of an adverse response to treatment at dose levels ≥20 mg/kg/day. As a result, the NOAEL was set at 4 mg/kg/day and the LOAEL at 20 mg/kg/day based on the aforementioned liver effects.

In addition to the revision to the subchronic oral dog study DER outlined above, new DERs were composed for guideline subchronic rat inhalation (MRID 50776901) and *in vitro*/*in vivo* dermal penetration studies (MRID 50776902) and a non-guideline acute oral study in dogs (MRID 46336401), and endpoints were selected for the inhalation and acute oral studies. The systemic and inhalation lowest-observed-adverse-effect-concentration (LOAEC) for the subchronic inhalation study could not be established due to a lack of adverse effects up to the highest concentration tested. The systemic and inhalation no-observed-adverse-effect-concentration (NOAEC) is 1.0 mg/L (achieved mean concentration of 1.08 mg/L). The LOAEL for the acute oral study in dogs is 200 mg/kg based on increased bilirubin in blood and urine, elevated liver enzymes, glycogen depletion, and bile stasis. The NOAEL is 100 mg/kg. Based on the data from the dermal penetration study, the *in vitro* dermal absorption for ethoxyquin is 34.1% in rats and 6.3% in humans, and the *in vivo* dermal absorption for ethoxyquin in rats is 41%. These values can be used to estimate dermal absorption for the ethoxyquin registration review risk assessment.

EPA Reviewer: Austin Wray **Signature:** Austin Wray
RABIV, Health Effects Division (7509P) **Date:** 09/09/19
EPA Secondary Reviewer: Jessica Kidwell **Signature:** for JK K Rickard
RABIV, Health Effects Division (7509P) **Date:** 09/09/19

Template version 03/12

TXR#: 0057939

DATA EVALUATION RECORD – Supplemental
See TXR # 0012357 for root DER

STUDY TYPE: Subchronic Oral Toxicity Study [capsule] – Dogs; OCSPP 870.3150 [§82-1b]; OECD 409.

PC CODE: 055501 **DP BARCODE:** D453267

TEST MATERIAL (PURITY): Ethoxyquin (98.2% a.i.)

SYNONYMS: 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline

CITATION: Naas, DJ (1996) A 90-Day Oral (Capsule) Toxicity Study of Ethoxyquin in Dogs. WIL Research Laboratories, Inc. Ashland, OH. Study No. WIL-273013, October 24, 1996. MRID 44148901. Unpublished.

SPONSOR: Oregon, Washington and California Pear Association, Portland, OR

EXECUTIVE SUMMARY:

In a subchronic oral toxicity study (MRID 44148901), beagle dogs (5/sex/group) were dosed with technical grade ethoxyquin (98.2% a.i.) via capsules at dose levels of 0, 2, 4, 20, or 40 mg/kg/day for 90 consecutive days. **Due to the systemic toxicity observed in the 40 mg/kg/day group, animals in this high dose group received the test material for only 7 weeks (49 days); these animals then remained on study for a six-week recovery period and received empty gelatin capsules.**

One female in the 40 mg/kg/day group was euthanized in extremis on study day 13 due to pneumonia. All other animals survived to the scheduled necropsy. Treatment-related clinical signs which were observed in 20 and 40 mg/kg/day groups (both sexes) consisted of brown areas of skin on the urogenital and ventral abdominal areas, brown sclera, discolored urine (brown or amber), decreased and/or black mucoid feces and/or emesis.

The 40 mg/kg/day group (both sexes) showed a decrease in body weight gain, or body weight loss, throughout the dosing period (7 weeks). At the end of the week 7, mean body weights at this dose were 14% and 22% lower than controls for males and females, respectively. During the recovery period, mean body weights in males were comparable to the control while females were still 14% lower than the control at week 13. In the 20 mg/kg/day group, although not statistically significant, mean body weights and body weight gains were lower than controls (3-10%) throughout the study. No effects on body weight were observed in the 2 and 4 mg/kg/day groups. See Table 1. The food consumption was markedly reduced in the 20 and 40 mg/kg/day groups throughout the dosing period. During the recovery period for the 40 mg/kg/day group, food consumption was comparable to the control level. The decreased food consumption may contribute to the body weight loss. No significant treatment-related effects were noted in the 2 and 4 mg/kg/day groups.

Hematology parameters did not show treatment-related effects. Clinical chemistry showed statistically significant increased levels of total bilirubin (3-5x above controls levels), alkaline phosphatase (ALP; 5.5-13.4x above control levels), alanine aminotransferase (ALT; 16.4-32.3x above control levels), aspartate aminotransferase (AST; 2.0-4.3x above control levels), and gamma glutamyl transferase (GGT; 2.8-13.7x above control levels) in the 20 and 40 mg/kg/day groups at week 4 and in the 20 mg/kg/day group at week 13. Liver enzyme levels in the 40 mg/kg/day group declined after exposure ended in week 7 but were still elevated above controls at week 13. In the 4 mg/kg/day group, although the group mean was not statistically significant, elevated ALP, AST and ALT levels were noted in one animal of each sex at week 4 and 13.

Macroscopic examination during necropsy showed dark livers in the 20 and 40 mg/kg/day groups (5 males and 4 females in each dose group). Microscopic examination revealed treatment-related microscopic lesions in the livers of the 4, 20, and 40 mg/kg/day groups. Bile duct hyperplasia was noted in 9/10 animals in the 20 mg/kg/day group (minimal to moderate severity) and 9/9 animals in the 40 mg/kg/day group (minimal to mild severity). Cytoplasmic vacuolation was seen in the liver of 3/10, 9/10, and 5/9 animals in the 4 (minimal severity), 20 (minimal to mild severity), and 40 mg/kg/day (minimal to moderate severity) groups, respectively. Minimal hepatocellular necrosis was noted in the 4 mg/kg/day group in a single male and a single female and in two males and one female in the 20 mg/kg/day group. The absence of hepatocellular necrosis and the decreased incidence and severity of cytoplasmic vacuolation and severity of bile duct hyperplasia in the 40 mg/kg/day group was likely due to the shorter period of dosing and subsequent recovery period for these animals. Endogenous pigmentation (hepatocytes and Kupffer cells) was observed in the livers of all animal groups with higher incidences observed in 20 and 40 mg/kg/day groups. The pigment was further identified as a porphyrin-class pigment.

The liver findings in the animals fed 4 mg/kg/day were not considered indicative of an adverse response to treatment. The liver microscopic findings were low incidence and minimal severity and the liver enzymes were only elevated in one animal/sex. Evidence for liver toxicity was more robust at dose levels $\geq$20 mg/kg/day. The liver effects were more diverse and included increases in additional liver enzymes and total bilirubin, and macroscopic changes. Furthermore, liver enzyme levels were elevated to a much larger degree compared to concurrent controls and the microscopic findings were observed with greater frequency and severity.