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June 9, 2015

Sent by email only to
P65Public.Comments@oehha.ca.gov and
Esther.Barajas-ochoa@oehha.ca.gov

Ms. Esther Barajas-Ochoa
Office of Environmental Health Hazard Assessment
PO Box 4010, MS-19B
Sacramento, CA 95812-4010

Re: Comments to Notice of Intent to List Chemicals by the Labor Code Mechanism: Aloe Vera, Whole Leaf Extract

Dear Ms. Barajas-Ochoa:

On behalf of Coats AgriAloe, LLC, successor to Coats Aloe International, Inc., we are pleased to provide our comments objecting to the Office of Environmental Health Hazard Assessment's ("Department's") proposed listing of Aloe Vera, Whole Leaf Extract ("Aloe Vera WLE"), as a chemical known to the State of California to cause cancer. These comments are submitted in response to the Department's Notice of Intent to List Chemicals by the Labor Code Mechanism (the "Notice") dated April 23, 2015. Thank you for the extension of time in which to submit comments.

We are addressing the following issues in our detailed comments below:

(a) by adopting the listing "whole leaf extract of Aloe vera" of the International Agency for Research on Cancer ("IARC") in Monograph 1089 (2015) ("Monograph"), the Department has failed to identify the specific chemical as a known or potential human or animal carcinogen

(b) by failing to specifically identify the chemical, the requirements of Health and Safety Code Section 25249.8(a), incorporating Labor Code section 6382((b)(1)) have not been met

(c) the proposed listing is defective and should be withdrawn

Failure to Identify the Specific Chemical Known to Cause Cancer

While we acknowledge, without agreeing, the Department's position that they cannot consider scientific arguments concerning the weight or quality of the evidence considered by IARC, we urge the Department to consider fully and carefully the contents of the IARC Monograph on Aloe Vera. As stated by IARC, "It is strongly recommended to consult the complete *Monographs* on these agents, the publication date, and the list of studies considered."[1] The data cited in support of the IARC listing of "whole leaf Aloe

[1] *http://monographs.iarc.fr/ENG/Classification/index.php*

vera" (the "Boudreau Study")[2] are inadequate as the basis for this listing. Indeed, even IARC stated that the study upon which they relied (see Boudreau Study below) as demonstrating "sufficient evidence in experimental animals for carcinogenicity" "would have been strengthened by the inclusion of positive controls".[3]

Composition of Aloe Vera WLE

IARC describes the Aloe Vera WLE as containing "both the gel from the inner parenchyma leaf pulp and the latex."[4] The contents of Aloe vera gel are dominantly glucose and mannose molecules and present no health concerns. See footnote 7. "Aloe vera latex contains four major C-glycosyl constituents: aloin A, aloin B, aloesin, and aloeresin A"[5] "In addition, the latex from Aloe vera contains several aromatic compounds, such as aldehydes and ketones[6]...The sugar moiety in aloins is D-glucose, and studies indicate that carbon atom 1 of the D-glucose moiety is linked directly to carbon atom 10 of the anthracene ring in a β-configuration...The carbon-carbon bond is quite resistant to acid and alkaline conditions; however, the intestinal microflora of humans and animals have been shown to cleave the β-C-glucosyl bond, although considerable variation in response among animal species occurs. Cleavage of the β-C-glucosyl bond results in the formation of aloe-emodin...."[7]

Further in the Monograph, IARC states "Aloe vera whole leaf extract is composed of gel and latex. Aloe vera gel contains non-starch polysaccharides of high molecular weight (the major one being acemannan) that are composed of sugar moieties linked by β-1,4-glycosyl bonds....Aloe vera latex contains the anthrone *C*-glycosides aloin A (barbaloin) and aloin B (isobarbaloin) that are linked by β-glycosyl bonds to D-glucopyranose. Other *C*-glycosides found in Aloe vera latex include aloesin (aloeresin B) and aloeresin A in which the glycosyl linkage is to the benzo ring of benzopyran-4-one....Aloenin, an O- β -glucoside, is also a component of Aloe vera latex...."[8]

Note that aloe-emodin is not listed in either section as a component of Aloe Vera WLE.

The listing of the chemicals found in the Aloe Vera WLE is revealing regarding the listing by IARC of Aloe vera, whole leaf extract, rather than the specific components. Section 3 of the Monograph discusses Cancer in Experimental Animals by considering only one study of oral administration in drinking water in mice and one study in rats, the Boudreau Study. The Boudreau Study is the sole basis of the IARC listing and it should be examined closely regarding the test material. IARC summarized the Boudreau Study test substance by stating "the average content of aloin A and aloe-emodin of the whole leaf test material was 6.40 and 0.071 mg/g respectively"[9]. **This statement is**

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[2] Boudreau MD, Beland Fa, Nichols JA, Pogribna M (2013a). Toxicology and carcinogenesis studies of a noncolorized whole leaf extract of Aloe barbadensis Miller (Aloe vera) in F344/N rats and B6C3F1 mice (drinking water study). NATL TOXICOL PROGRAM TECH REP SER, 577 (577):1-266. PMID: 24042237.

[3] IARC Monograph 108 (2015), page 27

[4] IARC, supra, page 5

[5] Ibid.

[6] Note that aldehydes and ketones are not usually considered aromatic compounds.

[7] IARC, supra, page 7

[8] IARC, supra, pg. 19

[9] IARC, supra, pg. 16

inaccurate and the inaccuracy implies that the Boudreau Study presented data regarding the aloe-emodin content of Aloe Vera WLE.

The Boudreau Study merely stated that for the 2-year study only, "Aloe-emodin was also assessed in nine randomly collected samples from *one lot of the blended and irradiated Aloe vera whole leaf extract.* The content of aloe-emodin was 70.5 ± _4.5 µg/g."[10] (emphasis added) However, even this limited statement was unsupported by the data presented in the study report. Unlike the levels of Aloin A and malic acid contained in the test materials, the Boudreau Study provided *no data* in Appendix I, Chemical Characterization and Dose Formulation Studies, in support of the assertion that one lot contained aloe-emodin. Regarding accuracy of these asserted measurements, the stability and recovery of the Aloin A was assessed in the test material, but there is no indication that either the stability or recovery of the aloe-emodin was assessed."[11] Further, only the irradiated Aloe vera whole leaf extract was tested for the presence of aloe-emodin. There are no data presented on un-irradiated Aloe vera whole leaf extract. The Peer Review Panel member Dr. Heiger-Bernays "...wondered whether the gamma irradiation of the test materials could modify their structures."[12] No discussion of the potential effect of irradiation was provided in the Boudreau Study.

In summary, the marker chemicals tracked in the Boudreau Study were malic acid and Aloin A, not aloe-emodin. No evidence of the aloe-emodin content of the test materials administered in the Boudreau Study was provided. See pages 37-38 of the Boudreau Study. There is insufficient evidence provided that the test material in the Boudreau Study contained an average of 0.071 mg/g aloe-emodin or that the aloe-emodin recovery and stability confirmed the accuracy of the analyses. Further, there is insufficient evidence that the Boudreau Study test materials were not affected by the irradiation of the aloe vera whole leaf extract. It is questionable whether the Boudreau Study test materials accurately represent Aloe vera WLE.

Formation of Aloe-Emodin

In contrast to whether aloe-emodin is present in the Aloe vera WLE, the IARC Monograph describes the formation of aloe-emodin as:

> Upon oral ingestion, Aloe vera components pass through the upper portion of the gastrointestinal tract; upon reaching the lower gastrointestinal tract, the anthrone *C*-glycosides aloin A and aloin B ***are converted by the intestinal microflora to aloe-emodin-9-anthrone, which undergoes sequential oxidation to aloe-emodin*** and rhein.[13] (emphasis added)

The Boudreau Study contains the following additional description of the difficulty of forming aloe-emodin in conditions outside of exposure to microflora within the intestinal system:

> The sugar moiety in aloins is D-glucose, and studies indicate that carbon atom 1 of the D-glucose moiety is linked directly to carbon atom 10 of the anthracene

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[10] Boudreau, supra pg. 36
[11] Boudreau, ibid.
[12] Boudreau, supra pg. 16.
[13] IARC, supra, pg. 28-29.

> ring in a β-configuration (Figure 2). ***The carbon–carbon bond is quite resistant to acid and alkaline conditions, and cleavage by oxidation, rather than hydrolysis, is achieved only under the drastic conditions of acid in combination with an oxidant*** (Hay and Haynes, 1956). ***The β-(1→1_0_)_ _C_–C bond is also resistant to β–glycosidase of plants and most plant bacteria*** (Vyth and Kamp, 1979; Joshi, 1998); ***however, the intestinal microflora of humans and animals have been shown to cleave the β-C-glucosyl bond, although considerable variation in response among animal species occurs*** (Mapp and McCarthy, 1970; Hattori *et al.*, 1988; Che *et al.*, 1991). ***Cleavage of the β-C-glucosyl bond results in the formation of aloe-emodin***, the cathartic principle of the latex, and other free anthraquinones and anthrones (Figure 2).[14] (emphasis added)

Significance of Aloe-Emodin

IARC specifically concluded in the Monograph that aloe-emodin, not Aloe Vera WLE, is the carcinogenic agent, whether acting alone or in combination with other materials.

> *Aloe vera* preparations, acemannan, and aloin A do not display genotoxic activity in bacterial assays for mutagenesis and/or other assays for genotoxicity. In contrast, aloe-emodin is mutagenic in *Salmonella typhimurium* reversion assays, induces unscheduled DNA synthesis, gene mutations, micronucleus formation, and chromosomal aberrations, inhibits topoisomerase II, and gives positive results in comet assays. These data suggest that the neoplastic response observed with *Aloe vera* is a consequence of the conversion of the anthrone *C*-glycosides to **aloe-emodin, which by itself or in combination with other Aloe vera components is responsible for the development of adenomas and carcinomas in the large intestine**."[15] (emphasis added)

However, this conclusion by IARC regarding aloe-emodin is unreferenced in the Monograph, unsupported by the referenced study, and inconsistent with a series of studies in which aloe-emodin demonstrated anticarcinogenic activity. See Attachment 1.

In addition, this conclusion by IARC regarding the carcinogenic effect of aloe-emodin is inconsistent with the Boudreau Study in which aloe-emodin was identified as the causative agent of the *cathartic, not carcinogenic,* effect.

> In plants, the majority of anthraquinones appear as anthraquinone O-glycosides, dianthrone O-glycosides, or, as in the case of Aloe vera, anthraquinone C-glycosides. Due to the β-glycosidic linkage between the sugar and the anthracene ring structure and the hydrophilic nature of the molecules, the anthraquinone C-glycosides in Aloe latex are protected, after oral administration, from acid hydrolysis in the stomach and enzymatic activity in small intestine and are carried unabsorbed to the large intestine of rats, where *Eubacterium* sp. act upon the C-glycoside anthranoids to release glucose and the free aglycone (Hattori *et al.*, 1993; van Gorkom *et al.*, 1999). ***Studies have shown that the cathartic effects of the Aloe latex are not due to the ingested form of the anthraquinone, aloin, but rather to the aglycone, aloe-emodin-9-anthrone, formed by bacterial metabolism of the aloin parent compound*** (Akao *et al.*,

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[14] Boudreau, supra, pg. 19
[15] IARC, supra, p. 29.

> 1996). The *Eubacterium* sp is expressed differentially across mammalian species; therefore, not all mammalian species are capable of transforming aloin to the aloe-emodin-9-anthrone (Werner, 2007; Canny and McCormick, 2008). ***In humans, the transformation of aloin to the purgative component, aloe-emodin-9-anthrone, is carried out by the intestinal anaerobe, Eubacterium sp. strain BAR*** (Che *et al.*, 1991; Hattori *et al.*, 1993; Akao *et al.*, 1996). [16] (emphasis added)

Summary

The IARC Monograph inaccurately identified the causative carcinogenic agent by listing of whole leaf extract of Aloe vera. Further, the IARC Monograph, inconsistent with listing of whole leaf extract of Aloe vera, identified, albeit without citation, the carcinogenic component of Aloe vera WLE as aloe-emodin. And finally, the IARC Monograph inappropriately relied upon the Boudreau Study as establishing that Aloe vera WLE contained aloe-emodin, which it did not.

Failure to Meet the Requirements of Health and Safety Code Section 25249.8(a), Incorporating Labor Code section 6382((b)(1)

The Department has proposed to list Aloe Vera WLE as a chemical known to the State of California to cause cancer under the requirements of Health and Safety Code section 25249.8(a) and Labor Code section 6382(b)(1). Section 25249.8(a) provides that the Department shall publish a list of chemicals known to cause cancer or reproductive toxicity and "such list shall include at a minimum those substances identified by reference in Labor Code Section 6382(b)(1)...." Labor Code 6382(b)(1) provides that the "listings referred to in subdivision (a) are as follows: (1) Substances listed as human or animal carcinogens by the International Agency for Research on Cancer (IARC)." Neither the Safe Drinking Water and Toxic Enforcement Act of 1986, nor the implementing regulations, define "chemicals". Labor Code section 6380 states that "[s]ubstances on the list shall be designated by their chemical and common name or names." Labor Code section 6367 defines "chemical name" as the "scientific designation of a substance in accordance with the nomenclature system developed by the International Union of Pure and Applied Chemistry or the system developed by the Chemical Abstracts Service." Labor Code section 6368 defines "common name" as "any designation or identification such as code name, code number, trade name, or brand name used to identify a substance other than by its chemical name. It is implicit under both the Proposition 65 and Labor Code provisions that the chemical identified by the State must be the specific chemical which is the causative agent. Section 25306(d)(2) of Title 27 CCR[17], in the context of the "authoritative bodies" mechanism for listing, makes this plain reading of the statute and regulations explicit: "(d) For purposes of this section a chemical is "formally identified" by an authoritative body when the lead agency determines that:...(2) the list, report, or document specifically and accurately identifies the chemical...."

As summarized in the discussion above, the Department's proposed listing of Aloe Vera WLE, based on the IARC's Aloe Vera Monograph, fails to accurately and

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[16] Boudreau, supra, pg. 92

[17] 27 CCR §25306(d)

sufficiently identify the chemical alleged to cause cancer. It is abundantly clear that Aloe Vera WLE contains a number of substances which are either benign or beneficial and which cannot be considered as meeting the criteria of "known to the State of California to cause cancer". The only substance alleged to cause cancer is a chemical formed by the metabolism of certain components of Aloe Vera WLE within the intestinal system of certain mammals. This substance, aloe-emodin, is not man-made nor within the control of manufacturers. Nor has the study on which IARC based their listing provided sufficient evidence to indicate that aloe-emodin is routinely present at measurable levels in Aloe Vera WLE. The Department's proposed listing of Aloe Vera WLE fails to specifically and accurately identify the chemical alleged to cause cancer.

Request to Withdraw Proposed Listing of Aloe Vera WLE as a Chemical Known to the State of California to Cause Cancer

For the reasons summarized above, we respectfully urge the Department to withdraw the proposed listing of Aloe Vera WLE as a chemical known to the state to cause cancer. While the Department may elect to not consider additional scientific evidence in making this decision, it is appropriate, as encouraged by IARC, to closely examine the study upon which the IARC listing was based. Close examination of that study indicates both deficiencies in the study as well as internal contradictions between the conclusions of the study and the cancer causing mechanism and substance. Even if Aloe Vera WLE contains aloe-emodin, which has not been established by the supporting study, listing Aloe Vera WLE as a chemical known to cause cancer would be analogous to listing tuna as a chemical known to cause cancer, rather than methylmercury. The listed substance does not accurately identify the alleged cancer causing agent.

We appreciate this opportunity to provide our comments. Please contact the undersigned if there are any questions.

Regards,

Deborah A. Chadbourne
Attorney for Coats AgriAloe, LLC

cc: Bill Coats, President, Coats AgriAloe, LLC

Comments to Notice of Intent to List Chemicals by the Labor Code Mechanism: Aloe Vera, Whole Leaf Extract

Attachment 1

Anticancer Activity of *Aloe vera Anthraquinones*

2000

Pecere T., Gazzola M.V., Mucignat C., Parolin C., Vecchia F.D., Cavaggioni A., Basso G., Diaspro A., Salvato B., Carli M., Palu G.: Aloe-emodin is a new type of anticancer agent with selective activity against neurodermal tumors. Cancer Research 60(11) 60(11):2800-2804, 2000.

2002

Wasserman L., Avibad S., Bery E., Nordenberg J., Febig E.: The effect of aloe emodin on the proliferation of a new merkel carcinoma cell line. Pathology 24(1):17-22, 2002.

2006

Lin J.G., Chen G.W., Li T.H., Chouh S.T., Ten T.W., Chung J.G.: Aloe emodin induces apoptosis in T24 human bladder cancer cells through P53 dependent apoptotic pathway. J. Urology 175(1):343-347, 2006.

2011

Ahirwar K., Jain S.K.: Aloe emodin novel anticancer herbal drug. International Journal of phytomedicine 3(1):27-31, 2011.

Tabolacci C., Oliverio S., Lentini A., Rossi S., Galbiati A., Montesano C., Mattioli P., Mattioli B., Facchiano F., Beninati S.: Aloe-emodin as antiproliferative and differentiating agent in human U937 monoblastic leukemia cells. Life Sciences 89(2):812-820, 2011.

Ahirwar K., Jain S.K.: Aloe-emodin novel anticancer herbal drug. Phytomedicine 3(1):27-31, 2011.

2012

Harley E., Nevo E., Lansky E.P., Ofir R., Bishagee A.: Anticancer potential of aloes: Antioxidant, Antiproliferative, and Immunostimulatory attributes. Planta Medica (2012-in press).

Suboj P.,, Babykutty S., Valiyaparambil G., Nair R.S., Srinivas P., Gopi D.R.: Aloe-emodin inhibits colon cancer cell migration/angiogenesis by down regulating MMP-2/9. Rho B and VEGF via reduced DNA binding of NF-kappa B. European J. of Pharmaceutical Sciences 45(5):581-591, 2012.

Suboj P., Babykutty S., Srinivas P., Gopala S.: Aloe-emodin induced G2/M/cell cycle and apoptosis via activation of caspase-6 in human colon cancer cells. Pharmacology 59(1-2):91-98, 2012.