October 17, 2016

Comments of
California League of Food Processors, California Retailers Association,
California Chamber of Commerce, California Grocers Association,
Western Agricultural Processors Association,
Grocery Manufacturers Association, and North American Meat Institute,
Regarding Whether
Nitrite in Combination with Amines or Amides
Has Been Clearly Shown Through Scientifically Valid Testing According to Generally Accepted Principles to Cause Cancer

I. Introduction

These comments regarding the potential listing of nitrite in combination with amines and amides are submitted to the Carcinogen Identification Committee (CIC or the Committee) on behalf of the California League of Food Processors, the California Retailers Association, the California Grocers Association, the Western Agricultural Processors Association, the California Chamber of Commerce, the Grocery Manufacturers Association, and the North American Meat Institute. These organizations represent thousands of California businesses large and small -- including farmers, growers, producers, restaurants, and retail sellers, among others -- which grow, produce, serve, sell, and use produce and other food products that contain nitrite in combination with an amine and/or an amide. These foods are a normal and necessary part of Californians' everyday lives.

The amorphous collection of combinations described as "nitrite in combination with amines or amides" includes thousands of possible combinations, some known and some unknown. Every plant-derived food contains amines and/or amides and the vast majority also likely contain detectable nitrite. These products include spinach, carrots, potatoes, kale, beets, eggplant, meat products, wine, beer, coffee, chocolate, and hundreds -- likely thousands -- more foods and beverages that will be covered if this collection of combinations is added to the list of chemicals known to cause cancer.

The Committee is asked to review "nitrite in combination with amines or amides." Proposition 65 assigns to this Committee the task of determining whether "a chemical is known to the state to cause cancer."[1] The CIC does so by rendering its opinion on whether "a chemical …has been clearly shown through scientifically

[1] Health & Saf. Code § 25249.8(b)

valid testing according to generally accepted principles to cause cancer." "Nitrite in combination with amines or amides" does not meet this standard.

The proposal that this ill-defined group of chemical combinations be considered for listing by the CIC presents an unusual set of circumstances, each one of which is rare. Taken together, listing in these circumstances would be unprecedented.

First, "nitrite in combination with amines or amides" is not "a chemical." The vast majority of substances on the list of "chemicals known to the state to cause cancer" are, in fact, "a chemical." The Proposition 65 list of carcinogens also contains a very small number of well-defined mixtures, or even a few specific products (*i.e.*, goldenseal root powder). But nitrite in combination with amines or amides is neither. Although nitrite is a single chemical,[2] the Committee is not asked to evaluate nitrite as a carcinogen, but to evaluate nitrite in combination with tens of thousands of unidentified amines and/or unidentified amides. We are unaware of any precedent for such a proposal or how such an undefined category could possibly meet the robust scientific standard the state's qualified experts require for listing a chemical as "known to cause cancer."

Second, the chemical combinations in these baskets are not identified. Neither the Office of Environmental Health Hazard Assessment (OEHHA), nor the Committee, nor the public, have a list of all the chemical combinations which fall under this heading, and it is unlikely one could be compiled. In preparing these comments, we reviewed the scientific and lay literature trying to find such lists. Not surprisingly, there were none, nor could we even find a list of all amines or all amides.

This is a significant problem for both regulated businesses, as well as for the regulators. We know, for example, that virtually all produce contain amines or amides and likely contain minute, but detectable amounts of nitrite; or they contain precursors that may break down into one or more of those chemicals when they are stored, handled, cooked or combined with other foods for consumption. Business owners would need organic chemistry degrees to know whether their products contain one or more of the thousands of possible amines or amides, and it is unlikely that no significant risk levels could be set or determined for more than a minute fraction of this total.

Third, the combinations of nitrite with amines and/or amides have not been clearly shown through scientifically valid testing to cause cancer. As set forth below in sections III (on toxicology) and IV (on genotoxicity), of the virtually limitless number of chemical combinations that would be listed as "known to the state to cause cancer" were nitrite in combination with amines or amides listed, only a handful have been tested for carcinogenicity. Of the 38 combinations tested for carcinogenicity in animals, the majority (24/38) of test results were negative. Even

[2]The Hazard Identification Document (HID) refers briefly to Nitrite "and its salts," but the chemical the CIC is asked to consider is "nitrite" and these comments are limited accordingly.

the reportedly positive animal tests for seven amines and seven amides in combination with nitrite may not withstand scientific scrutiny. More importantly, those reportedly positive results do not reflect a pattern or a mechanism of action that has application across the entire category of nitrite in combination with amines or amides or any significant subcategories. As we explain below in section V (on epidemiology), there is even less epidemiological support for listing nitrite in combination with amines or amides.

The CIC's responsibility is to determine whether "the chemical" has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer. For the overwhelming majority of chemical compounds and combinations that fall into the category of nitrite in combination with amines or amides, the scientific record falls far short of that standard. In fact, for most combinations, there is no scientific record. That alone should make it unnecessary to give any further consideration to this potential listing.

Fourth, as OEHHA explained on the first page of the HID summary, this "chemical" is not before the CIC because OEHHA concluded it should be listed; nor is it before the Committee because it was considered a priority chemical by the CIC. Rather, nitrite in combination with amines or amides is before the CIC because a peculiarity in the authoritative bodies regulation automatically routs it to the Committee's agenda.

A determination by the CIC is one method for adding a carcinogen to the list of chemicals "known to cause cancer." Another method is "if a body considered authoritative … has formally identified [the chemical] as causing cancer."[3] In 2014, OEHHA considered whether nitrite in combination with amines or amides should be listed using the authoritative bodies listing method, based on the International Agency for Research on Cancer's (IARC) review of that category. OEHHA concluded:

> "After consideration of comments received on the notice and further evaluation of the scientific evidence supporting the listing, OEHHA determined that the regulatory criteria in section 25306(e), Title 27 of the California Code of Regulations have not been met for the spectrum of chemicals covered by the broad class "nitrite in combination with amines or amides."[4]

Typically, once OEHHA proposes to list a chemical by the authoritative bodies mechanism, it does so. However, on the infrequent occasions it does not do so, the the Proposition 65 regulations mandate that the chemical be sent to the Committee.

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[3] Health & Saf. Code § 25249.8(b).

[4] See OEHHA's website at: http://oehha.ca.gov/proposition-65/crnr/nitrite-combination-amines-or-amides-be-considered-carcinogen-identification

> "[When OEHHA] finds that there is no substantial evidence that the criteria identified in [the regulations] have been satisfied, [OEHHA] shall refer the chemical to the … Committee to determine whether, in the Committee's opinion, the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity."[5]

In other words, once OEHHA determined that nitrite in combination with amines or amides did not meet the criteria for listing by the authoritative bodies mechanism, as happened here, OEHHA had no choice but to ask the CIC whether, in its opinion, the "'chemical'" nevertheless meets the CIC's own, more rigorous, scientific listing criteria.

Each of the four circumstances described above is relatively rare and may, alone suffice as a reason not to list nitrite in combination with amines or amides. That these four circumstances are present simultaneously here effectively rules out the prospect that nitrite in combination with amines or amides meets the listing criteria.

II. The only "chemical" and or combinations before the CIC *at this meeting* is the broad category of "nitrite in combination with amines and amides."

The CIC is asked to opine on whether a loose description of a massive number of chemical combinations should be added to the Proposition 65 list of carcinogens. The potential combinations covered by that description would substantially outnumber the entire existing Proposition 65 list of carcinogens and reproductive toxins. OEHHA has already determined that because IARC reviewed only a small number of chemical combinations, the authoritative bodies criteria for listing were not met for the "spectrum of chemicals covered by the broad class 'nitrite in combination with amines or amides." For that same reason, this broad and undefined collection of combinations has not been "clearly shown through scientifically valid testing according to generally accepted principles to cause cancer."

The question of whether *a specific combination*, *or a subset of combinations*, of nitrite with an amine or an amide has been clearly shown through scientifically valid testing to show cancer is *not* properly before the Committee *at this meeting*.

The only question before the CIC, and on which focused comments were solicited, is whether the entire category of nitrite in combination with amines or amides meets the criteria for being added to the list of chemicals known to the state to cause

[5] Cal. Code Regs., Tit.27 (Regs) §25306(i)

cancer. Any consideration *at this meeting,* of an individual combination, or a subset of combinations, is inappropriate and premature.

A. The HID document and the notice address the broad category of nitrite in combination with amines or amides, not any particular subcategory or individual combination.

The HID document is entitled "evidence on nitrite in combination with amines or amides." The announcement of the CIC's November 2016 meeting solicits comments on whether "nitrite in combination with amines or amides" should be listed.

> "OEHHA is referring "nitrite in combination with amines or amides" to the CIC for consideration for listing as causing cancer."[6]
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> "Nitrite in combination with amines or amides will be considered for possible listing by the CIC at its next meeting scheduled for Tuesday November 15, 2016."[7]

The HID and the notice of the CIC meeting do not identify for the public or for the CIC any subset or individual combinations the Committee or the public should specifically consider. As a result, neither the public nor the Committee's attention has been directed to any particular combination. The HID identifies animal tests for a total of 38 combinations, of which seven amine/nitrite and seven amide/nitrite combinations purport to be positive. But these results are cited as possible support for listing the broad category of nitrite in combination with amines or amides, not as evidence supporting a specific category or combination.[8] So, neither the Committee nor the public know whether their review of the scientific evidence should be limited or directed to those 14 individual combinations, to some other combinations, or to the whole spectrum. As is evident from the many fruits vegetables, and other foods that contain one or more of these combinations, there are compelling business and public health interests in hundreds of these combinations.

If the Committee is expected to consider a particular combination or subset of combinations for listing, providing adequate and specific notice of the particular combinations is necessary, not merely as a matter of procedural fairness; it is necessary to obtain the best scientific record. A review of the individual "positive" animal tests cited in the HID suggests that there may be deficiencies in even that

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[6] http://oehha.ca.gov/proposition-65/crnr/announcement-carcinogen-identification-committee-meeting-scheduled-november-15

[7] *Id.*

[8] "OEHHA developed this document with information on the evidence of 'nitrite in combination with amines or amides' to assist the CIC in its deliberations." HID, p. 8.

small number of tests, warranting more detailed analysis and illustrating the benefit of public comment on specific combinations if these are at issue.

B. Only the broad category "nitrite in combination with amines or amides qualifies to bypass the prioritization procedure for placing items before the CIC.

"Nitrite in combination with amines or amides" landed on the Committee's November 15 agenda by jumping the prioritization queue because of an anomaly in the Proposition 65 regulations. OEHHA explained on the first page of the HID, the sole reason "nitrite in combination with amines and amides" bypassed the normal prioritization process and is now before the CIC: "[T]hat broad spectrum of combinations was rejected for listing by the authoritative bodies mechanism." In other words, OEHHA found the proposed listing lacking using the authoritative bodies method, but it was required, under § 25306(i), to send that same proposed and rejected listing, "nitrite in combination with amines and amides," to the CIC. This jumping the line is a narrow exception to prioritization.

Significantly, OEHHA did not consider, and did not accept or reject for listing by the authoritative bodies method, any individual combination of chemicals or any subset of combinations. The only listing proposed using the authoritative bodies method is "nitrite in combination with amines and amides." That is, therefore, the only chemical combination that can bypass prioritization under § 25306(i).

The CIC's prioritization process was developed at the request of, and with the participation of, the CIC "to ensure that [its] efforts … are focused on chemicals that may pose significant hazards to Californians [and to provide] multiple opportunities for public input."[9] These multiple opportunities include the opportunity to comment on proper priority.

The prioritization process is, by its own terms, "the primary method by which a chemical can reach the CIC for consideration."[10] Exceptions to the process are specific and limited. The only one cited here is rejection by OEHHA of listing by the authoritative bodies listing method. That exception is limited to "*the* chemical" OEHHA *already considered and rejected* for listing by that method,[11] "nitrite in

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[9] *PROCESS FOR PRIORITIZING CHEMICALS FOR CONSIDERATION UNDER PROPOSITION 65 BY THE STATE'S QUALIFIED EXPERTS,* http://oehha.ca.gov/media/downloads/proposition-65/document/finalpriordoc.pdf p.1.

[10] *Id.* p.2.

[11] The regulation limits the burden placed on the CIC when a chemical is rejected for listing by the authoritative bodies process: The CIC's role is "to determine whether, in the Committee's opinion, *the* chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer." §25306(i) (Emphasis added).

combination with amines or amides" – not any other combination or subset of combinations.

If a subset of combinations is of interest to the Committee, it can of course instruct OEHHA to put that subset on the agenda for a future prioritization meeting. But consideration of a subset at this meeting is premature and, for the reasons described, would be without the benefit of appropriately noticed and focused public comment.

III. The animal studies do not clearly show through scientifically valid testing according to generally accepted principles that nitrite in combination with amines or amides causes cancer.

When OEHHA concluded that "nitrite in combination with amines or amides" could not be listed under the authoritative bodies listing method, it explained its decision.

> "Amines and [amides] are large classes of chemicals with thousands of individual members in each class. Because the animal studies cited by IARC as supporting the listing were based on a comparatively small number of chemicals, OEHHA determined in May 2015 that the regulatory criteria for listing via the authoritative bodies mechanism had not been met for the spectrum of chemicals covered by the broad class "nitrite in combination with amines or amides."[12]

For the same reason that OEHHA determined nitrite in combination with amines and amides could not be listed under the authoritative bodies listing mechanism, this broad class cannot be listed under the CIC's listing criteria (*i.e.*, the evidence is limited to a "comparatively small number of chemicals.").

A. Comparatively few amines have been shown to cause cancer in animals when given in combination with nitrite.

The HID concludes that "[f]or amines in combination with nitrite some animal bioassays report positive studies, while others do not."[13] Positive studies are defined as studies that show an increased incidence of tumors when an amine is given in combination with nitrite compared to when the amine alone, nitrite alone, and neither the amine nor nitrite are given. In fact, the vast majority of amines in combination with nitrite have not been shown to cause cancer in even a single animal study. Most of the possible combinations have never even been tested. (The same is true for amides in combination with nitrite, as will be shown in the next section.) To list nitrite in combination with amines as a broad category, it is

[12] OEHHA HID (2016) p. 1.

[13] *Id.*, p. 2.

necessary to conclude that amines in combination with nitrite have been clearly shown to cause cancer. The data simply do not support a conclusion that the entire class of amines in combination with nitrite has been clearly shown to cause cancer.

According to the HID, increased tumor incidences have been observed for at least one tumor type in at least one study for *only seven amines* in combination with nitrite; these seven amines are: aminopyrene, bis(2-hydroxypropyl)amine, chlorpheniramine, 2-amino-3-methylimidazo [4,5-f]quinolone (IQ), morpholine, N-methylaniline, and piperazine. In contrast, 16 other amines in combination with nitrite either did *not* increase the incidence of tumors or yielded inconclusive results; these 16 are: chlordiazepoxide, chlorpromazine, cimetidine, cyclizine, dibutylamine, dimethyl-dodecylamine, diphenhydramine, hepta-methyleneimine, hexamethylene-tetramine, lucanthone, methapyrilene, nitrilotriacetic acid, piperidine, propylhexedrine, PhIP, and trimethylamine. Further, the data also do not suggest there is a consistent pattern of carcinogenicity for any subclass of amines (*e.g.*, primary, secondary, tertiary, quarternary, cyclic).

According to the HID, "Primary amines represent a large class of hundreds of chemicals."[14] Yet, only two primary amines (*i.e.*, IQ and PhIP) in combination with nitrite were identified in the HID as having been tested for carcinogenicity in animals and only one primary amine (IQ) in combination with nitrite tested positive for carcinogenicity in animals. Even if both of these primary amines had tested positive for carcinogenicity, positive studies for only two primary amines in combination with nitrite would not be sufficient evidence to conclude that thousands of primary amines in combination with nitrite are clearly shown to cause cancer. In fact, it is very well known that primary amines do not react with nitrite to form N-nitrosamines.[15]

Similarly, the HID states, "There are also numerous secondary amines, of which eleven were tested in combination with nitrite in animals. Four tested positive … four tested negative and studies on three were inconclusive. Thirteen tertiary amines were tested, with three having some positive results … seven with negative results and three with inconclusive results." So, among the secondary and tertiary amines tested in combination with nitrite in animals, only seven out of 24 amines tested positive. One of the three tertiary amines with positive results in combination with nitrite is IQ, which is also identified in the HID as both a positive primary amine and cyclic amine.

There are no animal studies for the quaternary amines in combination with nitrite. Only five amines from the large class of cyclic amines were tested, and of these, only two showed positive results (*i.e.*, IQ and chlorpheniramine) in animal testing. The

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[14] *Id.*, p. 2.

[15] Collins CJ. Reactions of Primary Aliphatic Amines with Nitrous Acid, Acc. Chem. Res, 1971, 4(9), pp 315-322.

reaction chemistry of nitrite with all four classes of amines is significantly different. Nitrosamines are formed almost exclusively from secondary amines reacting with nitrite in a multi-step process.

The CIC is being asked to determine if nitrite in combination with amines is clearly shown to cause cancer on the basis of the results of seven amines out of thousands of combinations. Even the limited number of amines actually tested, were not chosen randomly. The investigators who conducted these animal studies chose only those amines they considered most likely to cause cancer in animals in combination with nitrite. Thus, the subset of 23 amines tested in combination with nitrite cannot be considered representative of the thousands of combinations of nitrite and amines. Even so, most (16 out of 23) of the comparatively few tested combinations of nitrite and amines did not produce positive carcinogenic results in animal studies.

B. Comparatively few amides have been shown to cause cancer in animals when given in combination with nitrite.

As in the case of some amines described above, some amides in combination with nitrite produced positive carcinogenicity results in animals, while others did not. According to the HID, only seven out of the 15 amides tested have been shown to increase the incidence of tumors in animals when given in combination with nitrite. These seven include: allantoin, butylurea, carbendazim, ethylene thiourea, ethylurea, methylurea, and dodine. Yet, according to the HID, another eight amides in combination with nitrite did not produce positive results. Furthermore, thousands of other amides have never been evaluated in combination with nitrite for evidence of carcinogenicity in animals.

Given the comparatively small number of amides in combination with nitrite with positive carcinogenicity results, it cannot be concluded that the entire class of amides in combination with nitrite has been clearly shown to cause cancer. Even without any negative studies, it would not be possible to extrapolate the results with seven amides across the broad class of amides. However, given there are more negative studies than positive studies of amides, there is no way to conclude that the broad class of nitrite in combination with amides have been clearly shown to cause cancer. As in the case of amines, it is likely that the amides selected for animal testing in combination with nitrite were not chosen randomly. It is more likely they were chosen by the study authors because they believed these specific amides offered the greatest potential to cause cancer in animals when administered in combination with nitrite. Yet, fewer than half of these amides in combination with nitrite produced positive carcinogenicity results.

In summary, nitrite in combination with amines or amides does not meet the CIC's listing criteria for the same reason OEHHA determined the proposed listing does not meet the authoritative bodies criteria (*i.e.*, the evidence is limited to a

"comparatively small number of chemicals."). Even among the relatively small number of examples of amines and amides selected for carcinogenicity testing in combination with nitrite, there was no consistent evidence of carcinogenicity. Some were characterized in the HID as having positive evidence of carcinogenicity; others were not. These results do not satisfy the "clearly shown" to cause cancer standard.

C. Among the relatively small number of combinations characterized in the HID as positive for producing cancer in animals, not all of these combinations exhibit sufficient evidence of carcinogenicity.

The HID identifies only a relatively small number of combinations as positive for animal carcinogenicity. Specifically, seven amines and seven amides in combination with nitrite are characterized in the HID as positive for animal carcinogenicity, as indicated by a "Yes" in Tables 7 and 8. This list of combinations is very small compared to the thousands of possible combinations; moreover, analysis of even those limited combinations shows the number, if any, that have been "clearly shown" to cause cancer is even smaller.

For example, chlorpheniramine, an anti-histamine drug, was evaluated in combination with nitrite in male and female F344 rats in a single study by Lijinsky (1984).[16] In male rats, chlorpheniramine in combination with nitrite produced a statistically significant increase in liver tumors compared to groups given chlorpheniramine alone, nitrite alone and the negative control (*i.e.*, given neither chlorpheniramine nor nitrite). However, there was no significant increase in tumors of the pituitary, forestomach, pancreas, adrenal medulla, mammary glands or leukemia among male rats given the combination of chlorpheniramine and nitrite compared to the other three groups.

In female rats, administration of chlorpheniramine in combination with nitrite did not produce a statistically significant increase in any type of tumor compared to the groups given chlorpheniramine alone, nitrite alone, and the negative control group. Thus, there was no evidence of carcinogenicity among female rats given chlorpheniramine in combination with nitrite in the sole animal study conducted on this combination.

It is unclear whether this study would qualify as "scientifically valid testing." For example, the group size was 24 males and 24 females per group. The recommended group size for 2-year carcinogenicity studies is at least 50 males and 50 females per group. Also, this study employed only a single dose of chlorpheniramine and a single dose of nitrite. Therefore, it is not possible to evaluate whether a dose-response relationship exists.

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[16] HID (2016) Table 7, p. 97.

In summary, the evidence of carcinogenicity of chlorpheniramine in combination with nitrite in animals is limited to one tumor type (liver) in one sex (males) in one species (rats) in one study (which is unlikely to meet the requirement of "scientifically valid testing."). Notwithstanding these several limitations, this example is characterized in Table 7 of the HID as one of the seven positive combinations.

IV. The genotoxicity data do not clearly show through scientifically valid testing according to generally accepted principles that nitrite in combination with amines or amides cause cancer.

The HID identifies 111 amines and 39 amides that have been tested in combination with nitrite for genotoxicity. The vast majority of the genotoxicity tests are *in vitro* studies. Similar to the results of the animal carcinogenicity studies, some of the genotoxicity studies of amines and amides in combination with nitrite report positive findings, while others do not.

Out of the 111 combinations of amines and nitrite tested, the HID identifies 59 amines, a little more than half, in combination with nitrite with at least one positive genotoxicity test.[17] The HID considered a positive test to be one where a greater effect was observed with the combination than with nitrite alone and with the amine or amide alone. Among the 39 amides tested for genotoxicity in combination with nitrite, the HID identifies 15 amides, less than half, in combination with nitrite that tested positive.[18] Importantly, the HID reminds the Committee: "However, the overall number of amines and amides tested for genotoxicity still represent a small portion of the thousands of individual amines and amides that exist."[19]

The data in the HID yield other important insights, as well. First, and most importantly, only a relatively small subset of combinations of nitrite and amines or amides were even tested. Among those tested in combination with nitrite, only 53% of the amines and only 38% of the amides tested positive (defined as at least one positive genotoxicity study).

Second, there are more examples of nitrite in combination with amines or amides tested for genotoxicity than tested for carcinogenicity in animals. Among the 59 amines with positive evidence of genotoxicity, only ten were evaluated in combination with nitrite for carcinogenicity in animals, and only five (aminopterin, ethylurea, methylurea, morpholine, piperazine) were characterized in the HID as positive for carcinogenicity in animals. In other words, among the 59 amines that

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[17] *Id.* p. 113.

[18] *Id.*

[19] *Id.* p. 178.

tested positive for genotoxicity in combination with nitrite, 54 have not been shown to be carcinogenic in animals. Evidence of genotoxicity in the absence of evidence of carcinogenicity in humans or animals is not enough to conclude that a substance has been clearly shown to cause cancer.

Third, in the case of many of the combinations identified as positive for genotoxicity in the HID, only a single genotoxicity test (usually an Ames test) was performed. Thus, it is not possible to fully evaluate the weight of the evidence of genotoxicity based on a single assay or test.

Finally, for certain combinations, the evidence of genotoxicity was limited to positive results in a single study -- even though multiple studies were performed. For example, cimetidine (also known as Tagamet) in combination with nitrite was evaluated for genotoxicity in five *in vitro* or *in vivo* studies. Only one test (an Ames test in the TA1535 strain) was identified as a positive genotoxicity test in the HID. The other four tests did not yield such a result.

It is important to recognize that the amines and amides selected for testing for genotoxicity in combination with nitrite were not chosen randomly. Rather, they were selected because they were among the most likely candidates to be highly nitrosatable and to form potentially genotoxic N-nitroso compounds. Nevertheless, roughly half of the tested combinations were not demonstrated to be genotoxic. The genotoxicity studies of a limited number of combinations with varying results do not provide sufficient evidence to conclude that nitrite in combination with amines or amides has been clearly shown to cause cancer.

V. The epidemiological studies do not clearly show through scientifically valid testing according to generally accepted principles that nitrite in combination with amines or amides cause cancer.

A. Overview of Epidemiologic Evidence

Based on the epidemiologic evidence, nitrite in combination with amines or amides does not meet the CIC's listing criteria for three reasons. First, no epidemiologic studies have evaluated nitrite in combination with amines or amides. Thus, there is no human epidemiologic evidence to support an association between this type of exposure and a risk of cancer. Furthermore, it may be methodologically impossible to ascertain all sources of nitrite exposure and all sources of amines or amides as an interactive variable (*i.e.*, the synergistic relationship between nitrite with amines/amides on cancer risk).

Second, although nitrite, nitrate, or a combination of nitrite and nitrate have been evaluated as independent variables in numerous prospective cohort and case-control studies, the epidemiologic evidence does not support an increased risk between nitrite and/or nitrate and any type of cancer. Some positive associations between

dietary nitrite exposure (estimated based on self-reported food intake) and gastric cancer have been reported in early case-control studies that have well-known methodological issues, namely, the high likelihood of recall bias and selection bias. However, over the past six years, some large prospective cohort studies of higher methodological quality have been published that provide no support for an increased risk of gastric cancer based on dietary nitrite intake.

Third, a wealth of epidemiologic studies have reported decreased risks of many types of cancer in analyses of food groups (*e.g.*, fruits and vegetables) and specific food categories (*e.g.*, green leafy vegetables) that are sources rich in nitrite. In summary, based on a critical appraisal of the epidemiologic evidence, there is no support for a conclusion that dietary intake of nitrite in combination with amines or amides increases the risk of any type of cancer and no basis for concluding dietary nitrite in combination with amines or amides plays a causal role in human cancer development.

B. No epidemiologic studies have evaluated nitrite in combination with amines or amides.

The HID has recapitulated the associations between dietary nitrite (and nitrate in some instances) and risk of several types of cancer across the human epidemiologic studies. However, this body of epidemiologic literature does not include findings for nitrite *in combination with* amines or amides -- because such analyses do not exist.

The HID devotes 59 pages to the section entitled "Carcinogenicity Studies in Humans," but does not identify a single epidemiological study of nitrite in combination with amines or amides.[20] The HID describes dozens of epidemiological studies of nitrite, but none of those studies involve nitrite in combination with amines or amides. The HID's Executive Summary discusses the epidemiological studies on page 2.

> "Many studies are available examining cancer in humans in relation to ***nitrite*** intake. Some studies report positive associations, and some do not. Evidence of carcinogenicity comes primarily from cohort and case-control studies of colorectal, esophageal and stomach cancer [in relation to nitrite intake]. IARC (2010) evaluated the evidence from studies in humans and concluded: 'There is limited evidence in humans for the carcinogenicity of ***nitrite*** in food. ***Nitrite*** in food is associated with increased incidence of stomach cancer.'" [21] [Emphasis added]

Conspicuous in its absence from this discussion of epidemiological data is any mention of amines or amides. Nor does the summary mention nitrite in

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[20] *Id.* p. 24-83.

[21] *Id.* p. 2.

combination with amines or amides. The issue before the CIC regarding this proposed listing is not nitrite. It is whether nitrite in combination with amines and amides has been clearly shown to cause cancer.

The Committee should not draw the erroneous conclusion that epidemiological studies of nitrite are really studies of nitrite in combination with amines and amides because all humans are exposed to amines and amides in food. To evaluate nitrite in combination with amines or amides, it is not enough to quantify exposure to nitrite. In order to assess the carcinogenic potential of the combination, it is critical that exposure to both nitrite and amines or amides be determined and there are no epidemiological studies that meet this test.

In assessing the animal studies, the HID goes to great lengths to define the experimental groups required to determine whether there is positive evidence of carcinogenicity of nitrite in combination with amines or amides in animals. According to the HID, four groups are required: (1) a negative control group (neither nitrite nor amine or amide), (2) a nitrite only group, (3) an amine or amide only group, and finally (4) a nitrite plus amine or amide group. The HID did not consider an animal study to provide positive evidence of the carcinogenicity of nitrite in combination with amines or amides unless the study included all four groups. The HID also did not consider an animal study to be positive if any one of these groups was missing.

Using this standard, there are no epidemiological studies that come close to meeting the requirements for a properly designed study of nitrite in combination with amines or amides. There are no epidemiologic studies that have ascertained dietary nitrite plus amines or amides from the totality of the human diet as an exposure variable. Therefore, there are no epidemiologic studies that compare populations with high exposure to dietary 'nitrite plus amines or amides' with populations with low exposure to dietary 'nitrite plus amines or amides' and risk of cancer. There are certainly no epidemiologic studies that compare populations with dietary exposure to nitrite in combination with amines or amides to populations who are ***not*** exposed. It is inconceivable that a study population is not exposed to nitrite in combination with amines or amides from dietary sources.

On a daily basis, human beings are exposed to nitrites, amines, and amides from numerous dietary sources. Broadly, these products include a wide variety of vegetables, fruits, meats, cheeses, grains, coffee, and alcoholic beverages. Because of this breadth, it may be methodologically impossible to ascertain all sources of nitrite exposure and all sources of amines or amides as an interactive variable (*i.e.*, the synergistic relationship between nitrite with amines/amides on cancer risk) that can be examined in epidemiologic studies. Furthermore, humans may be exposed to amines or amides from vitamin supplements, dyes, and over-the-counter and prescription pharmaceuticals among other sources. In summary, it is not possible

to draw a conclusion that nitrite in combination with amines or amides causes cancer in humans.

C. The epidemiologic evidence is not supportive of a causal relationship between dietary nitrite or nitrate and cancer.

Although the issue before the Committee is neither nitrite nor nitrate, it is reassuring that the epidemiologic evidence is not supportive of a causal relationship between dietary nitrite or nitrate or cancer. Although nitrites in combination with amines or amides have not been analyzed specifically in observational or human clinical trials, the potential relationship between ingested nitrite and nitrate and the risk of cancer has been examined in a large volume of epidemiologic studies spanning study populations across several geographic regions. Cancer outcomes typically associated with diet given their anatomical and physiological relevance, such as colorectal, esophageal, and gastric cancer, have been investigated most extensively regarding dietary nitrate and nitrite intake. Other cancer sites, such as those in the central nervous system and thyroid, and malignancies of lymphohematopoietic origin, have limited epidemiologic data from high quality prospective cohort studies for ingested nitrate and nitrite.

The HID appears to rely largely upon the 2010 IARC evaluation, where that body concluded: "There is limited evidence in humans for the carcinogenicity of nitrite in food. Nitrite in food is associated with increased incidence of stomach cancer." IARC defines limited evidence in humans as the observation of a positive association between exposure to an agent and cancer for which chance, bias or confounding cannot be ruled out with reasonable confidence.

The potential relationship between dietary nitrite exposure and stomach cancer has been investigated in more epidemiologic studies than other types of cancer. This is likely due to the fact that positive associations were reported in early case-control studies (with well-known methodological deficiencies, namely the high likelihood of recall bias and selection bias), which may have prompted IARC to conclude in 2010 that "Nitrite in food is associated with increased incidence of stomach cancer." However, since the 2010 IARC assessment, some large prospective cohort studies of higher methodological quality compared with the previous case-control studies have been published that provide no support for an increased risk of stomach cancer based on dietary nitrite intake.[22,23,24] In fact, two of these studies[22,23] observed non-

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[22] Cross AJ, Freedman ND, Ren J, Ward MH, Hollenbeck AR, Schatzkin A, Sinha R, Abnet CC. Meat consumption and risk of esophageal and gastric cancer in a large prospective study. The American Journal of Gastroenterology. 2011; 106:432-442.

[23] Loh YH, Jakszyn P, Luben RN, Mulligan AA, Mitrou PN, Khaw KT. N-Nitroso compounds and cancer incidence: the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study. The American Journal of Clinical Nutrition. 2011; 93:1053-1061.

statistically significant decreased risks of gastric cancer based on the highest exposure categories of nitrate and nitrite intake.

Recently, Xie and colleagues (2016)[25] published a comprehensive meta-analysis of dietary nitrate and nitrite intake and several different types of cancer, but focused on studies of gastric, esophageal, and colorectal cancers. Overall, they observed weakly elevated, non-statistically significant summary associations of 1.20, 1.25, and 1.12 for nitrite exposure and gastric, esophageal, and colorectal cancers, respectively. Importantly, the summary associations, albeit already weak in magnitude, attenuated to nearly null or non-statistically significant decreased risks after meta-analyzing the sub-groups of studies that adjusted for physical activity, body mass index, cigarette smoking, and alcohol consumption. All of these variables clearly confounded the overall association between nitrite exposure and these cancer outcomes. Nitrate exposure was associated with a statistically significant decreased risk of gastric cancer. Epidemiologic evidence from prospective cohort studies of nitrate and/or nitrite and other types of cancer are limited, thus, reliable conclusions cannot be drawn at the present time.

Based on a critical appraisal of the epidemiologic evidence, there is no support for a conclusion that dietary intake of nitrate and/or nitrite increases the risk of any type of cancer. Insofar as the epidemiologic data are not supportive of an increased risk of cancer, there is no basis for a conclusion that dietary nitrate and/or nitrite plays a causal role in human cancer development. This interpretation is relatively consistent with the 2010 IARC determination of limited evidence in humans for some cancer sites, whereby chance (*i.e.*, unable to distinguish an observed association from one that is null or due to a chance finding), bias, or confounding cannot be ruled out. However, a more accurate determination for most types of cancer would be "inadequate evidence of carcinogenicity" or "evidence suggesting lack of carcinogenicity" in human populations.

D. There is no clear evidence of human cancer risk based on epidemiologic studies of food groups or individual food sources of nitrites, amines, or amides.

The human diet involves (as it should) exposure to nitrites, amines, and amides from numerous dietary essentials, many of which are nutrient dense sources of vitamins, minerals, and antioxidants. These include, but are not limited to, a wide variety of vegetables, fruits, meats, cheeses, and grains. Methodologically, constituents of food intake, such as nitrite, are not captured on food frequency

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[24] Keszei AP, Goldbohm RA, Schouten LJ, Jakszyn P, van den Brandt PA. Dietary N-nitroso compounds, endogenous nitrosation, and the risk of esophageal and gastric cancer subtypes in the Netherlands Cohort Study. The American Journal of Clinical Nutrition. 2013; 97:135-146.

[25] Xie L, Mo M, Jia H, Liang F, Yuan J and Zhu J (2016). Association between dietary nitrate and nitrite intake and site-specific cancer risk: evidence from observational studies. Oncotarget: 1-18

questionnaires or other dietary assessments in epidemiologic studies. Rather, exposure is typically estimated based on the content of the constituent within the food or the likelihood of exposure using food composition databases. Because of this, it is difficult to evaluate the independent relationship between a constituent, such as nitrite, and cancer risk. It is of even greater methodological difficulty to evaluate nitrite in combination with other constituents that are not specific to an individual food source, such as amines or amides.

Food groups and specific foods that are rich sources of nitrites, amines, or amides have been examined across a voluminous body of epidemiologic studies. Indeed, decreased risks of many types of cancer in analyses of these food groups and specific foods have been reported in large prospective cohort studies and meta-analyses. For example, in two systematic meta-analyses of cruciferous vegetable consumption by Wu and colleagues (2013)[26,27], statistically significant 19% and 18% decreased risks were observed based on 22 studies of gastric cancer and 35 studies of colorectal cancer, respectively. In a large prospective cohort study of approximately 500,000 U.S. men and women, total fruit and vegetable intake was associated with a 22% decreased risk of squamous cell carcinoma of the esophagus.[28] Collectively, evaluations of food groups or specific foods that are rich sources of nitrites and/or amines/amides are not supportive of increased risks of human cancer.

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We respectfully submit that the scientific evidence presented shows:

1) A relative absence of toxicological testing;
2) Mixed results for the limited toxicological data that do exist; and
3) A complete absence of applicable epidemiological evidence.

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[26] Wu QJ, Yang Y, Wang J, Han LH, Xiang YB. Cruciferous vegetable consumption and gastric cancer risk: a meta-analysis of epidemiological studies. Cancer Sci. 2013 Aug; 104(8):1067-73

[27] Wu QJ, Yang Y, Vogtmann E, Wang J, Han LH, Li HL, Xiang YB. Cruciferous vegetables intake and the risk of colorectal cancer: a meta-analysis of observational studies. Ann Oncol. 2013 Apr; 24(4):1079-87

[28] Freedman ND, Park Y, Subar AF, Hollenbeck AR, Leitzmann MF, Schatzkin A, Abnet CC. Fruit and vegetable intake and esophageal cancer in a large prospective cohort study. Int J Cancer. 2007 Dec 15; 121(12):2753-60.

For all the above reasons, nitrite in combination with amines or amides has not been clearly shown to cause cancer.

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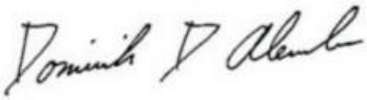

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For

California Food Processors Association
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