Exposure Estimate for Semicarbazide from the Use of Azodicarbonamide in Bread for the U.S. Population

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Introduction

Azodicarbonamide (ADA) is approved for use (21 CFR 172.806):

- As an aging and bleaching ingredient in cereal flour in an amount not to exceed 2.05 grams per 100 pounds of flour (0.0045 percent; 45 parts per million).
- As a dough conditioner in bread baking in a total amount not to exceed 0.0045 percent (45 parts per million) by weight of the flour used.

During bread baking, ADA breaks down to form biurea, semicarbazide (SEM), and urazole. The formation of SEM has raised health concerns and prompted the U.S. Food and Drug Administration (FDA) to conduct an analysis to determine which food products use ADA as an ingredient, and therefore, which products are mostly likely to contain SEM, and the levels at which SEM is present in food.

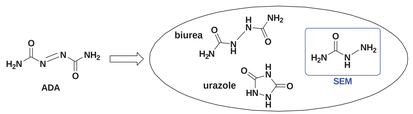

Figure 1: Thermal decomposition of ADA during baking forms biurea, semicarbazide (SEM), and urazole.

A survey of food products in the U.S. using Mintel's Global New Products Database (GNPD) indicated that ADA is primarily used as an ingredient in bakery products. Further analysis of products in the bakery category indicated that 79 % of the food products in this category that list ADA as an ingredient are bread and bread products.

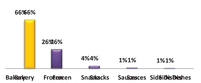

Figure 2: Food Products Containing ADA

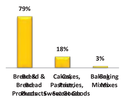

Figure 3: Bakery Products Containing ADA

Figure 4: Percentage of Bread & Bread Products Containing ADA by Year.

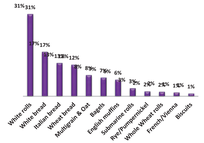

Figure 5: Relative Use of ADA in Bread & Bread Products by Food Category.

Focusing on the consumption of bread and bread products, the FDA conducted an exposure estimate for SEM for the U.S. population aged 2 years and older and children aged 2-5 years using 2-day and 10-14 day food consumption data.

Identification of Bread Products Containing ADA as an Ingredient

Two different sources were used to identify bread products labeled as containing ADA:

- FoodEssentials LabelBase is a proprietary product label database providing access to information from greater than 250,000 food labels compiled from the Gladson and Mintel databases for U.S. products. This database was used to identify the food products that list ADA as an ingredient. [Gladson Nutrition Database contains information from over 90% of products in most major consumer packaged goods categories. It includes information such as product images, ingredient lists, nutrition facts, and universal product codes (UPC).] Mintel GNPD monitors product innovation and retail success in the consumer packaged goods market. It contains similar data for 62 countries in 32 food categories from 1996 to the present. This database was also used to identify the food products that list ADA as an ingredient.
- Product Label Survey: Data collected from the FoodEssentials LabelBase were verified by conducting a survey of product labels at local grocery stores, bakeries, and fast food restaurants in various states including Arizona, Arkansas, California, Georgia, Illinois, Maryland, Minnesota, Missouri, New York, Ohio, Pennsylvania, and Texas during 2013.

Source of SEM Use Levels in Foods

- Analytical data: Based on the product label survey, approximately 250 representative products were chosen by FDA for analysis for SEM by a contract laboratory.
- A variety of commercial products were purchased from food stores, bakeries, and fast food restaurants. The products included bagels, biscuits, breadsticks, cinnamon & raisin bread, croutons, English muffins, frankfurter rolls, focaccia bread, French bread, garlic bread, hamburger rolls, Italian bread, multigrain breads & rolls, oat bread, potato bread, pumpernickel bread & rolls, reduced calorie bread, rye bread & rolls, sourdough bread, submarine rolls, stuffing, white bread & rolls, whole wheat bread & rolls, Vienna bread, and various sweet rolls and pastries, including donuts.
- A peer-reviewed method using liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) developed by FDA's Office of Regulatory Science (ORS) with a limit of detection of 5 µg/kg was used in this study.[1] The level of SEM determined in a given food product was provided in µg/kg.

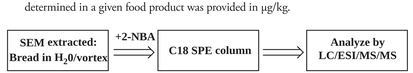

Figure 6: Schematic of sample preparation procedure.

- Prior to analysis, all products were prepared as they would be consumed. The samples were then extracted with water, and SEM derivatized with o-nitrobenzaldehyde (2-NBA) prior to LC/MS/MS analysis for $[M+H^+]$ m/z 209.1→166 (15 eV)/134 (17 eV)

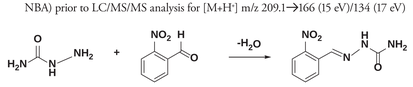

Figure 7: Derivatization of SEM using o-nitrobenzaldehyde (2-NBA).

(1) Noonan, G. O.; Warner, C. R.; Hsu, W.; Begley, T. H.; Perfetti, G. A.; Diachenko, G. W. *J. Agric. Food Chem.* **2005**, *53*, 4680.

Method of Estimating Exposure

Dietary exposure estimates for SEM from the use of ADA in bread and bread products were performed using two different sets of food consumption data: 1) the combined 2009-2012 National Health and Nutrition Examination Survey (NHANES) 2-day dietary intake survey; and 2) the 2007-2010 NPD Group, Inc. National Eating Trends-Nutrient Intake Database (NPD NET-NID) 10-14 day data using the Foods Analysis and Residue Evaluation-National Eating Trends (FARE-NET) program.

Two population groups were chosen for the exposure estimate:

- U.S. population aged 2 years and older
- Children aged 2-5 years

The NPD NET-NID data provide food frequency data from 10-14 day food diaries for over 5,000 respondents. The FARE-NET program provides food consumption data based on the food frequency data from the NPD NET-NID combined with the mean food intakes from NHANES.

All dietary exposures were estimated on an "eaters-only" basis, meaning that the estimate represents the dietary exposure to SEM from the consumption of bread and bread products containing SEM by the individuals in the population who consumed one or more of those foods over the survey period. Dietary exposures were estimated at the mean and the 90th percentile, where the 90th percentile represents those individuals who are the "high intake" consumers of a given food.

The exposure estimate was performed as follows:

- Foods that could contain SEM were identified based on label information for ADA. These foods were then grouped into 15 broad food categories.
- Over 500 NHANES food codes were assigned to these food categories.
- For each NHANES food code, the use level of SEM, based on the results of the analytical data, was assigned to that food code. If a given food code did not contain ADA as an ingredient, then that food code was not included in the exposure estimate.
- For the exposure estimate using NPD NET-NID data (10-14 day food consumption data), the NHANES food codes were mapped to the NPD NET-NID food codes.
- For each broad category, three different exposure scenarios were performed:
  - ► Low Exposure Scenario: The lowest analytical value for SEM for a given bread or bread product was assigned to each food code. This represents the lowest exposure to SEM.
  - ► Average Exposure Scenario: The analytical results were averaged for a given food code.
  - ► High Exposure Scenario: The highest analytical value for SEM for a given bread or bread product was assigned to each food code. This represents the highest exposure to SEM.
  - ► For those food codes where only one product was represented by a given food code, the same SEM level was used for all three scenarios.
- For each population group, the following eaters-only exposures to SEM were estimated:
  - ► Each individual food category of bakery products.
  - ► Cumulative exposure for all breads, including French or Vienna bread, Italian bread, multigrain & oat bread, rye & pumpernickel bread, wheat bread, white bread, and other breads, which include potato and sweet potato breads.
  - ► Cumulative exposure for all rolls, including white rolls, wheat rolls, and submarine rolls.
  - ► Cumulative exposure that takes into account all food categories considered in the exposure estimate.

Results

Table 1: Eaters-only Exposure to SEM (µg/p/d) for the U.S. Population Aged 2 Years and Older Based on 2009-2012 NHANES 2- Day Food Consumption Data.

	Food Category	% Eaters	Low Exposure Scenario Mean	Low Exposure Scenario 90th Percentile	Average Exposure Scenario Mean	Average Exposure Scenario 90th Percentile	High Exposure Scenario Mean	High Exposure Scenario 90th Percentile
U.S. Population Aged 2 Years and Older	Bagels	8	2	6	3	6	4	6
	Biscuits	8	0.2	0.3	4	7	22	43
	Stuffing†	5	0.1	0.4	2	6	9	30
	Muffins, English	4	9	17	12	18	15	24
	Donuts††	13	7	18	9	22	10	25
	Bread, French or Vienna	3	3	5	17	27	38	70
	Bread, Italian	7	0.7	2	3	7	7	17
	Bread, Multigrain & Oat	6	0.2	0.4	7	13	12	24
	Bread, Rye & Pumpernickel	2	0.2	0.3	2	5	9	18
	Bread, Wheat	27	0.6	1	2	5	3	9
	Bread, White	29	0.3	1	3	6	13	27
	Bread, Other	0.4	10	20	11	23	12	25
	All Breads	**62**	**0.7**	**2**	**4**	**9**	**12**	**27**
	Roll, Submarine	7	8	13	41	67	74	121
	Roll, Wheat/ Grain	3	0.4	1	7	10	14	28
	Roll, White	37	0.3	0.2	5	9	33	60
	All Rolls	**43**	**2**	**7**	**11**	**34**	**41**	**88**
	Cumulative	**87**	**3**	**10**	**11**	**27**	**34**	**79**

†Includes Dressings & Croutons; ††Includes Sweet Rolls, Pastries, and Croissants.

Table 2: Eaters-only Exposure to SEM (µg/p/d) for the U.S. Population Aged 2 Years and Older Based on NPD NET-NID 10-14 Day Food Consumption Data.

	Food Category	% Eaters	Low Exposure Scenario Mean	Low Exposure Scenario 90th Percentile	Average Exposure Scenario Mean	Average Exposure Scenario 90th Percentile	High Exposure Scenario Mean	High Exposure Scenario 90th Percentile
U.S. Population Aged 2 Years and Older 10-14 day Food Consumption Data	Bagels	23	n/a	7	n/a	7	n/a	7
	Biscuits	51	n/a	n/a	2	9	11	23
	Stuffing†	26	n/a	1	1	9	6	13
	Muffins, English	18	4	10	4	10	5	10
	Donuts††	40	2	9	2	9	3	9
	Bread, French or Vienna	9	n/a	4	3	9	8	17
	Bread, Italian	39	n/a	1	1	9	2	9
	Bread, Multigrain & Oat	8	n/a	1	2	9	3	10
	Bread, Rye & Pumpernickel	11	n/a	n/a	1	7	3	10
	Bread, Wheat	51	n/a	3	1	9	2	9
	Bread, White	91	n/a	2	2	9	10	21
	Bread, Other	2	3	9	4	10	4	10
	All Breads	**97**	**1**	**9**	**4**	**9**	**13**	**27**
	Roll, Submarine	68	3	9	13	28	24	50
	Roll, Wheat/ Grain	14	n/a	1	1	8	2	9
	Roll, White	88	n/a	7	3	9	19	38
	All Rolls	**94**	**2**	**9**	**12**	**28**	**35**	**69**
	Cumulative	**100**	**4**	**10**	**18**	**36**	**56**	**99**

n/a: not applicable due to low percentage of eaters; †Includes Dressings & Croutons; ††Includes Sweet Rolls, Pastries, and Croissants.

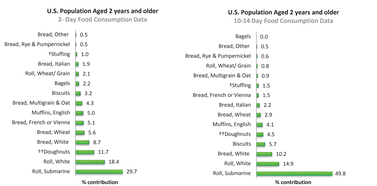

Figure 7: Relative percent contribution to SEM exposure by food category for the mean average exposure scenario for the U.S. population aged 2 years and older using 2-day and 10-14 food consumption data. (†Includes Dressings & Croutons; ††Includes Sweet Rolls, Pastries, and Croissants.)

Table 3: Eaters-only Exposure to SEM (µg/p/d) for Children Aged 2-5 Years Based on 2009-2012 NHANES 2-Day Food Consumption Data.

	Food Category	% Eaters	Low Exposure Scenario Mean	Low Exposure Scenario 90th Percentile	Average Exposure Scenario Mean	Average Exposure Scenario 90th Percentile	High Exposure Scenario Mean	High Exposure Scenario 90th Percentile
Children Aged 2-5 Years 2-day Food Consumption Data	Bagels	7	1	4	1	4	2	6
	Biscuits	7	0.1	0.2	3	6	14	40
	Stuffing†	2	0.1	0.2	2	4	7	17
	Muffins, English	3	3	12	6	13	8	14
	Donuts††	13	4	13	6	15	7	17
	Bread, French or Vienna	2	1	3	10	17	25	44
	Bread, Italian	6	0.5	1	2	4	3	7
	Bread, Multigrain & Oat	4	0.2	0.3	4	7	7	12
	Bread, Rye & Pumpernickel	0.1	n/a	0.1	1	n/a	5	n/a
	Bread, Wheat	33	0.4	1	1	4	2	7
	Bread, White	32	0.2	0.3	2	4	10	19
	Bread, Other	1	7	10	8	12	9	13
	All Breads	**64**	**1**	**1**	**3**	**6**	**8**	**17**
	Roll, Submarine	1	3	5	16	26	28	46
	Roll, Wheat/ Grain	2	0.3	1	3	5	8	14
	Roll, White	28	0.1	0.1	3	4	19	30
	Roll, White	28	0.1	0.1	3	4	19	30
	All Rolls	**31**	**0.2**	**0.2**	**3**	**6**	**19**	**33**
	Cumulative	**82**	**1**	**3**	**5**	**11**	**16**	**35**

n/a: not applicable due to low percentage of eaters; †Includes Dressings & Croutons; ††Includes Sweet Rolls, Pastries, and Croissants.

Table 4: Eaters-only Exposure to SEM (µg/p/d) for Children Aged 2-5 Years Based on NPD NET-NID 10-14 Day Food Consumption Data.

	Food Category	% Eaters	Low Exposure Scenario Mean	Low Exposure Scenario 90th Percentile	Average Exposure Scenario Mean	Average Exposure Scenario 90th Percentile	High Exposure Scenario Mean	High Exposure Scenario 90th Percentile
Children Aged 2-5 years 10-14 day Food Consumption Data	Bagels	17	n/a	2	n/a	2	n/a	2
	Biscuits	40	n/a	n/a	1	8	6	15
	Stuffing†	23	n/a	n/a	1	2	2	8
	Muffins, English	10	2	9	2	9	3	9
	Donuts††	39	1	8	1	9	2	9
	Bread, French or Vienna	8	n/a	1	1	5	4	9
	Bread, Italian	42	n/a	1	n/a	4	1	9
	Bread, Multigrain & Oat	4	n/a	n/a	1	4	2	7
	Bread, Rye & Pumpernickel	3	n/a	n/a	n/a	1	2	4
	Bread, Wheat	54	n/a	1	1	4	2	7
	Bread, White	95	n/a	1	2	9	10	19
	Bread, Other	2	2	7	2	8	2	8
	All Breads	**98**	**n/a**	**7**	**3**	**9**	**11**	**21**
	Roll, Submarine	46	1	5	7	15	12	25
	Roll, Wheat/ Grain	13	n/a	1	1	2	2	5
	Roll, White	81	n/a	n/a	1	6	10	20
	All Rolls	**87**	**1**	**6**	**5**	**13**	**16**	**33**
	Cumulative	**100**	**2**	**9**	**8**	**18**	**29**	**51**

n/a: not applicable due to low percentage of eaters; †Includes Dressings & Croutons; ††Includes Sweet Rolls, Pastries, and Croissants.

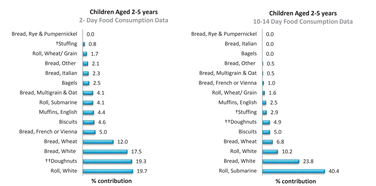

Figure 8: Relative percent contribution to SEM exposure by food category for the mean average exposure scenario for children aged 2-5 years using 2-day and 10-14 food consumption data. (†Includes Dressings & Croutons; ††Includes Sweet Rolls, Pastries, and Croissants.)

Analytical Data

Table 5: Range of SEM Analytical Results (µg/kg) for Bread and Bread Products Considered in the Exposure Estimate

Bagels	<5	-	117	Submarine Rolls	35	-	1260
Biscuits	<5	-	767	Wheat Breads	<5	-	191
English Muffins	<5	-	466	Wheat & Grains Rolls	<5	-	657
French or Vienna Breads	40	-	1490	White Breads	<5	-	368
Italian Breads	<5	-	606	White Rolls	<5	-	862
Multigrain & Oat Breads	<5	-	341	††Donuts	<5	-	384
Other Breads	299	-	366	†Stuffing	<5	-	368
Pumpernickel & Rye Breads	<5	-	315				

†Includes Dressings & Croutons; ††Includes Sweet Rolls, Pastries, and Croissants.

Summary

- Eaters-only dietary exposure to SEM was estimated for each individual food category of bakery products, a cumulative exposure for all breads, a cumulative exposure for all rolls, and a total cumulative exposure for all the categories considered containing SEM for the U.S. population aged 2 years and older and children aged 2-5 years using 2-day and 10-14 day food consumption data.
- The use of 2-day food consumption data to assess chronic exposure can lead to an overestimation of exposure, especially for certain types of breads that are not commonly consumed. For this reason, data collected from a 10-14 day survey are considered to be more representative of actual consumption patterns in the U.S.
- The highest exposure was estimated for white rolls, white bread, and submarine rolls.

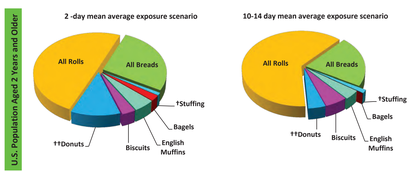

Figure 9: Cumulative mean average exposure estimate to SEM (µg/p/d) for all breads, all rolls, and other bread-based products for the U.S. population aged 2 years and older using 2-day and 10-14 day consumption data. (†Includes Dressings & Croutons; ††Includes Sweet Rolls, Pastries, and Croissants.)

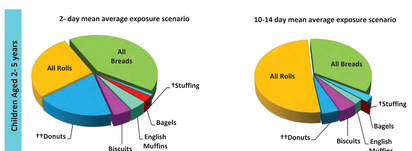

Figure 10: Cumulative mean average exposure estimate to SEM (µg/p/d) for all breads, all rolls, and other bread-based products for children aged 2-5 years using 2-day and 10-14 day consumption data. (†Includes Dressings & Croutons; ††Includes Sweet Rolls, Pastries, and Croissants.)