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October 9th, 2015

Wim Debeuckelaere
Head of Sector for Additives, Flavorings, Enzymes
DG SANTE – Directorate-General for Health and Consumer Protection
European Commission
Rue Breydel 4
1049 Brussels, Belgium

Re: Purity of Indigo carmine

Dear Mr Debeuckelaere,

I am writing on behalf of the International Association of Color Manufacturers (IACM) with regards to the recent Scientific Opinion published by the ANS Panel on the safety of indigo carmine as a food color and the Comments that IACM submitted to the Panel to which you were also copied (letter dated July 1st 2015, appended).

In their response to our comments, the ANS Panel reiterated that it overruled the adverse effects seen in this study and did not consider the study of adequate quality to change the current ADI of 5 mg/kg bw/day. However, the Panel has restricted its safety determination of indigo carmine to material of purity > 93% due to the uncertainty introduced by the Dixit and Goyal study.

IACM and our members take the safety of their products very seriously. As stated in our comments, the material used in the Dixit and Goyal, 2013 study that showed signs of toxicity in male mice was of questionable source (ASES Chemical Works[1]). No information on the specifications and quality of the sample were provided in the published study, nor were specifications requested by the Panel for the purpose of its safety evaluation of the color. Therefore, it is not known whether the coloring substance tested in this study was of food grade quality or other grade coloring material.

By contrast the food grade indigo carmine that our members place in the market is material of consistent quality that meets food safety regulatory standards. In the USA, indigo carmine is "batch-certified" by the US-FDA. That is, every batch of food grade color undergoes chemical analysis at US-FDA laboratories to confirm compliance with standards of purity and other specifications. The minimum

[1] http://ases.co.in/prodetail.aspx?id=634&pname=INDIGO%20CARMINE&sid=0&ftype=&flag=I%25&product_code=

purity standard accepted internationally for food grade indigo carmine is 85% of coloring matter. Most batches produced by our member companies are consistently of purity closer to 90% or higher, owing to reproducible manufacturing processes according to GMP standards.

Our members have high confidence in the quality of their products and were very disappointed that material of unknown quality would be used as the basis for setting purity criteria for the safety of food grade indigo carmine. Following the ANS Panel Opinion, our members made significant efforts to obtain a sample of the material that was tested in the above mentioned study for the purpose of performing a full chemical analysis and comparing it to representative materials that are approved for use as food colors. The results of such analysis were recently submitted to IACM and we wish to bring these data to your attention and hope that they are taken into consideration in the process of any risk management decisions. The analytical results are presented in the Table at the end of this letter.

The results of the analysis (Table 1) demonstrate the following deviations about the unknown material tested in the Dixit and Goyal, 2013 study (ASES product) compared to Lot AW3535, a batch-certified material provided kindly by one of IACM's member companies:

1- The ASES product does not meet the current minimum purity standard of 85%
2- It contains almost twice as much sodium chloride (NaCl) and over 6 times as much sodium sulfate (Na_2SO_4)
3- The level of the subsidiary dye, Isatin-5-sulfonic acid, is 12-fold higher in the ASES product compared to the Lot AW3535 and exceeds the US standard by more than 4-fold
4- The level of the subsidiary dye, 5-sulfoanthranilic acid, in the ASES product is more than twice as high as the USA standard (not detectable in the Lot AW3535).
5- The sum of the two subsidiary dyes listed above in the ASES product exceeded the EC limit by more than 4-fold.
6- The level of isomeric dye was minimal in the ASES product although it is expected to be detected under US standards up to 18%
7- The total percent (%) of trisulfonated dyes in the ASES product was 470 times higher than in the Lot AW3535
8- Of the heavy metals, cadmium levels in the ASES product were 2.3-fold higher than the EC limit (and 23-fold higher than the Lot AW3535); lead was 4 times higher in the ASES product compared to the Lot AW3535, although both were below the allowed limits.

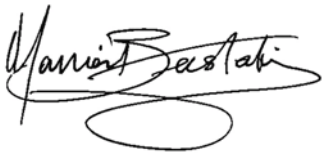
Taken together, the above results amply demonstrate that the test material used in the Dixit and Goyal, 2013 study is very different compared to the material produced by manufacturers who supply food grade indigo carmine in the EU market and other regions and it does not meet food grade specifications under any regulatory jurisdiction. Therefore, the adverse effects reported in this study as a result of ingestion of this dye cannot be considered as valid indication of the safety of food grade indigo carmine, produced under high quality standards and in compliance with existing safety provisions and regulations.

IACM members are committed to high quality standards for their products. Attached you may also find the Certificates of Analysis for representative batches of Indigo Carmine as provided by two other manufacturers. Our color manufacturers are also committed to provide samples for comparative testing by the EC or EFSA at an independent laboratory of their choice.

IACM would be pleased to provide relevant information about food color quality and safety in support of EFSA safety evaluations as it has demonstrated in the recent years with response to request of information for a number of food colors. It is our desire that risk management decisions are made upon reliable, scientifically defensible evidence. We hope that the information provided in this letter is taken into consideration in this process.

Sincerely,

International Association of Color Manufacturers (IACM)
Maria Bastaki, Ph.D.
Scientific Director



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Reference

Dixit A and Goyal RP, 2013. Evaluation of Reproductive toxicity caused by Indigo carmine on male swiss albino mice. Pharmacology OnLine, 1, 218–224.

Table 1 – Results of Chemical Analysis of Indigo Carmine samples relative to regulatory standards

Food grade and US-FDA Certified lot from an IACM member company compared to a sample lot from ASES Chemical Works, India.

		Samples analyzed		Regulatory authority					Differences	
Test Description	**Alternate Chemical name**	**Lot AW3535**	**ASES Chem.Works Lot 065**	**U.S.** FD&C Blue No. 2	**E.C.** E 132 Indigotine Indigo Carmine	**JECFA** INS 132 Ingidotine Indigo Carmine	**Japan** Food Blue No. 2	**China** GB 28317-2012	**Fold difference ASES/ Lot AW3535**	**Fold difference ASES/Reg.Limit**
Pure dye (by Spectrophotometric analysis (%))		**91.16**	**82.48**	≥85.0%	≥85.0%	≥85%	≥85.0%	≥85.0%	0.90	0.97
NaCl (%)		**2.00**	**3.56**						1.78	
Na_2SO_4 by I. C. (%)		**1.57**	**9.80**						6.24	
Loss on Drying (Volatile Matter-Oven (%), Moisture)		4.58	4.04	--	--		≤10.0%		0.88	
Chloride & Sulfate (As sodium salts)		3.57	13.36	--	--	--	≤7.0%		3.74	
Moisture and Salts		8.15	17.40	≤15%	--	≤15%	--	≤15%	2.13	
Water Insoluble Matter (%)		0.01	0.14	≤0.4%	≤0.2%	≤0.2%	≤0.20%	≤0.20%	14	
Subsidiary Dyes				≤1.0%	--	≤1.0%	--	--		
Isatin-5-sulfonic acid (%)	Isatin-5-sulfonic acid	0.14	1.66	≤0.4%	--	--	--	--	11.86	4.15
5 sulfoanthranilic acid (%)	5-sulfoanthranilic acid	0.00	0.45	≤0.2%	--	--	--	--		2.25
Isomeric (%)	Disodium salt of 2-(1,3-dihydro-3-oxo-7-sulfo-2H -indol-2-ylidene)-2,3-dihydro-3-oxo-1H -indole-5-sulfonic acid	**14.73**	**0.24**	≤18%	--	--	--	--	0.02	

		Samples analyzed		Regulatory authority					Differences	
Test Description	**Alternate Chemical name**	**Lot AW3535**	**ASES Chem.Works Lot 065**	**U.S.** FD&C Blue No. 2	**E.C.** E 132 Indigotine Indigo Carmine	**JECFA** INS 132 Ingidotine Indigo Carmine	**Japan** Food Blue No. 2	**China** GB 28317-2012	**Fold difference ASES/ Lot AW3535**	**Fold difference ASES/Reg.Limit**
Lower Sulfonated (%)	Sodium salt of 2-(1,3-dihydro-3-oxo-2H -indol-2-ylidene)-2,3-dihydro-3-oxo-1H -indole-5-sulfonic acid	0.00	0.47	≤2%	--	--	--	--		
Trisulfonated (%)		**0.01**	**4.70**						470	
Sum of Isatin-5-sulfonic Acid, 5-sulfoanthranilic acid and Anthranilic acid*		**0.14**	**2.11**	--	**≤0.5%**	--	--	--	15.1	4.22
Unsulfonated primary aromatic amines (as aniline)				--	≤0.01%	≤0.01%	--			
Ether Extractable matter				**--**	≤0.2%	≤0.2%	--			
Metals										
Lead (ppm)		**0.1**	**0.4**	≤10	≤2	≤2	--	≤10	4	
Arsenic (ppm)		0.1	0.1	≤3	≤3	--	≤4 (as As_2O_3)	≤1	1	
Mercury (ppm)		0.1	0.1	≤1	≤1	--	--	--	1	
Cadmium (ppm)		**0.1**	**2.3**	--	**≤1**	--	--	--	23	2.3

*Anthranilic acid not tested.

FDA Comments

LOT NO: AW3535 FD&C BLUE No. 2
Sensient Colors LLC
Batch No.: 5303513 Weight: 2,998.26 LBS JUL-01-2015

DETERMINATION	METHOD	FOUND	UNIT	DATE
TARTRATE BUFFER TITRATION	1	91	%	JUN-25-2015
SPECTROPHOTOMETRIC	2	91.16	%	JUN-25-2015
VOLATILE MATTER	3	4.58	%	JUN-25-2015
NACL	4	2	%	JUN-25-2015
NA2SO4 BY I. C.	5	1.57	%	JUN-25-2015
WATER INSOLUBLE MATTER	6	0.01	%	JUN-26-2015
ISATIN-5-SULFONIC ACID	64	0.14	%	JUL-01-2015
ISATIN-7-SULFONIC ACID	176	NR	%	JUL-01-2015
ISOMERIC	65	14.73	%	JUL-01-2015
LOWER SULFONATED SUBSIDIARY	66	NF	%	JUL-01-2015
TRISULFONATED	175	0.04	%	JUL-01-2015
MERCURY	7	PT	ppm	JUN-26-2015
LEAD	8	PT	ppm	JUN-26-2015
ARSENIC	9	PT	ppm	JUN-26-2015

CALLUP DUPLICATE - NOT OFFICIAL COPY
Food and Drug Administration Analytical Results
61 20155417 FORM FDA 3000 OMB NO. 0910-0216 (10/31/2001) 8220