DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Washington, DC 20204

JUL - 9 1998

Mr. Michael A. Zeligs
President
BioResponse, L.L.C.
P.O. Box 288
Boulder, Colorado 80306-0288

Dear Mr. Zeligs:

2235 '98 JUL 10 P2:29 P2:29

This letter acknowledges your withdrawal of your August 11, 1997 new dietary ingredient submission to the Food and Drug Administration for diindolylmethane (DIM) and your letter dated June 9, 1998, enclosing information to support your contention that DIM was marketed as a dietary ingredient in the United States before October 15, 1994, and therefore is not a new dietary ingredient under 21 U.S.C. 350b(c). the information you have provided will be filed in Docket No. 95S-0316 to complete the record for your submission.

Please contact us if you have any questions concerning this matter.

Sincerely,

James T. Tanner, Ph.D.
Acting Director
Division of Programs and Enforcement Policy
Office of Special Nutritionals
Center for Food Safety
and Applied Nutrition

Copies:
FDA, Center for Food Safety and Applied Nutrition, Imports Branch, HFS-606
FDA, Office of the Associate Commissioner for Regulatory Affairs, Division of Imports Operations and Policy, HFC-170
FDA, New York District Office, Imports Branch, HFR-NE150

cc:
HFA-224 (w/incoming)
HFA-305 (docket 95S-0316)
HFS-22 (CCO)
HFS-456 (file, r/f)
HFS-450 (r/f, file)
GCF-1 (Nickerson)
reviewed/verbal concurrence:GCF-1:7/1/98
f/t:rjm:HFS-456:7/9/98:zeligs2.ltr:disc30

95S-0316

SUP2

BioResponse L.L.C.
P.O.Box 288
Boulder, CO 80306-0288

June 9, 1998

James Tanner, Ph.D., Acting Director
Robert Moore, Ph.D.
Michael Bolger. Ph.D
SueAnn Assimon, Ph.D.
Division of Programs and Enforcement Policy
Office of Special Nutritionals
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 "C" Street, S.W., HFS-455
Washington, D.C. Delivered Via Federal Express

Dear CFSAN staff,

Thank you for acknowledging our meeting request so rapidly and for the informative meeting held May 29, 1998. At your request, an affidavit to document that DIM is not a "new dietary ingredient" is provided. Also attached is further clarification of information presented at the meeting concerning the history of human use of Diindolylmethane (DIM).

We have enclosed the requested affidavit under Appendix I. Designed Nutritional Products, Inc. manufactured and sold 100 kg of DIM prior to October 15, 1994, and the President, David Parish, has provided this affidavit attesting to the manufacture and sale of DIM for use in dietary supplements.

During a discussion of the new information BioResponse sent to CFSAN on May 18, 1998, it was noted that the design of the survey that was used to collect human use data for DIM and I3C was not clearly stated. To clarify this history of human use, details of this survey of individuals with Recurrent Respiratory Papillomatosis (RRP), along with additional responses and more readable charts are included in Appendix II. Based on this survey of human use of excessive doses of DIM and I3C, no side effects were identified in humans ingesting DIM at up to 14 mg/kg/day for 12 months and I3C as high as 21 mg/kg/day for up to 32 months.

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Based on comments from FDA regarding the greater strength of physician supplied information, and to augment the data from the Rosen paper (see Appendix 2 of the new information sent May 18, 1998), BioResponse contacted 2 physicians directly supervising I3C supplementation. These responses from physicians following and evaluating RRP patients on I3C supplementation are summarized in Appendix III. These patients have been adding encapsulated I3C to their diet at doses in excess of 5 mg/kg/day. This inquiry revealed a further subset of 115 individuals ingesting I3C capsules on a chronic basis under medical supervision.

Finally, with regard to the central issue of enzyme induction and activity possibly associated with supplemental use of dietary indoles, findings supporting the unique role of DIM as a direct inhibitor of both the Aryl Hydrocarbon Receptor (AhR) and CYP1A enzymes are reviewed in Appendix IV. This summary of the meeting presentation by Dr. David Williams again differentiates DIM from other dietary indoles.

Once again we would like to thank you for the rapid review and the opportunity to discuss new information supporting DIM safety at the May 29, 1998 meeting.

Sincerely,

Michael A. Zeligs, M.D.
President, BioResponse

Telephone 303 447-3841
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Appendix I

This appendix contains the affidavit and supporting documents regarding the manufacturing, labeling, and sales of DIM prior to October 15, 1994.

Affidavit from David Parish, President, Designed Nutritional Products, Inc.

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Facsimile 303-938-8003

DESIGNED NUTRITIONAL PRODUCTS

P. O. Box 1242 OREM, UT 84059-1242
Telephone (801) 224-4518 FAX (801) 226-8496

AFFIDAVIT

BY OVERNIGHT COURIER

June 9, 1998

Robert J. Moore, Ph.D.
Regulatory Branch (HFS 456)
Division of Programs and Enforcement Policy
Office of Special Nutritionals
Center for Food Safety and Applied Nutrition
U.S. Food and Drug Administration
Room 4129C
Federal Office Building #8
200 C Street, S.W.
Washington, D.C. 20204

Dear Mr. Moore:

I understand from Ms. Elizabeth T. Zeligs of BioResponse, L.L.C., Boulder Colorado, that she and other representatives of BioResponse, L.L.C. met with you and other representatives of the U.S. Food and Drug Administration (FDA) on May 29, 1998, and at that time you requested a notarized affidavit from Designed Nutritional Products to provide documentation for FDA that the dietary ingredient diindolylmethane (DIM) is not a new dietary ingredient. (Another name for this compound is: bis-(3-indolyl)methane.) This Affidavit responds to that request.

I am advised that the Dietary Supplement Health and Education Act (DSHEA) provides that a "new dietary ingredient" means a dietary ingredient that was not marketed in the United States before October 15, 1994 and does not include any dietary ingredient which was marketed in the United States before October 15, 1994". Title 21, United States Code (U.S.C.), section 350b(c).

Given this definition, it is clear that DIM is not a new dietary ingredient because our company marketed the substance in the United States for use as a dietary ingredient in dietary supplement products before October 15, 1994. The attached papers document this fact. To the best of my knowledge the foregoing information is true and accurate.

Respectfully submitted,

David Parish
President
Designed Nutritional Products

06-09-98
Date

Attachment: Letter dated May 14, 1998 to Elizabeth Zeligs, Bio Response, L.L.C., From Merrill Andrus, Designed Nutritional Products, including the five (5) exhibits that accompanied that letter.

State of Utah)
SS:
County of Utah)

On this 9th **day of** June **19** 98 **, before me** Barbara J Edge **, a notary Public, personally appeared** David Parish **, personally known to be the person(s) whose name(s) is (are) subscribed to this instrument, and acknowledged that he (she) (they) executed and sworn an oath, saying the foregoing is a true statement.**

S E A L

BARBARA J EDGE
Notary Public
State of Utah
My Comm. Expires Jul 14, 2000
163 S 1140 E Lindon UT 84042

Barbara J Edge
Notary Public

My Commission Expires on 7-14-2000

DESIGNED NUTRITIONAL PRODUCTS

P. O. Box 1242 OREM, UT 84059-1242
Telephone (801) 224-4518 FAX (801) 226-8496

14 May 1998

Elizabeth Zeligs
BioResponse L. L. C.
P. O. Box 288
Boulder, CO 80306-0288 FAX # (303) 938-8003

Dear Mrs. Zeligs:

There is no doubt that bis-(3-indolyl) methane was prepared, isolated, characterized, and then incorporated into dietary supplements and marketed prior to 15 October 1994. This product has been known by a number of names such as: 3,3'-diindolyl methane, Diindolylmethane, DIM, BIM, "dimer," etc.

Discussions and plans concerning dietary supplements containing dietary indoles were conducted in 1989. It was known then that indole-3-carbinol, bis-(3-indolyl) methane, and ascorbigen were dietary indoles which could be found in such natural sources as the juices of cabbage, broccoli, and Brussels sprouts.

By 1993 the three dietary indoles: indole-3-carbinol, bis-(3-indolyl) methane, and ascorbigen, each had been synthesized at Parish Chemical Company and mixtures of two or three were combined with other ingredients to make dietary supplements which were promoted and sold in the dietary supplement industry. As shown above bis-(3-indolyl) methane has had a number of other names applied to it and any of those names can be misspelled in a given plant document.

Two particular products involved:

1. Mixed dietary indoles called Indoles on Broccoli.

2. A complete vitamin C containing dietary indoles called "C plex."

Unfortunately, a disastrous fire at Parish Chemical Company in July of 1992 destroyed records of the earliest transactions. Following the fire business conditions were in disarray for many months. However, we have located:

A. a purchase order dated 2 Sep 1993

B. a tablet composition report dated 21 Sep 1993

C. a manufacturing batch record dated 25 Oct 1993

D. a certificate of analysis for the manufacture of 3,3' bis-indolylmethane dated 2 February 1994.

E. a certificate of manufacture dated 3 November 1995 showing that bis-(3-indolyl) methane is a customary ingredient in the 25% indoles mixture product.

Items A, B, C, and E all apply to synthesized indoles blended into broccoli powder so that the mixture has been sometimes as low as 3.8%, sometimes up to 25% indoles as sold to manufactures who incorporated the mixture into tablets or capsules.

Items A and B give some idea of the proportion of bis-(3-indolyl) methane as compared to indole-3-carbinol incorporated into the mixtures. In those instances the bis-(3-indolyl) methane was about 17% of the total indoles.

I have interviewed the people who were involved in production activities from 1990 to October 1994. According to their recollection the production of bis-(3-indolyl) methane was on a laboratory scale until early in 1994. In January and February an number of small batches in the plant produced a total of about 100 kilograms. The Item D mentioned above is the Certificate of Analysis for one of these batches. All of that material was incorporated into indoles blended on broccoli powder or in a vitamin C mixture which was variously called C plete and C Curity. All of these products were sold to manufacturers of dietary supplements.

I believe this information and these exhibits will satisfy your most pressing needs. This letter with its attachments will be sent to you by mail.

Sincerely,

G. Merrill Andrus
DESIGNED NUTRITIONAL PRODUCTS

ENHANCED LIVING INTERNATIONAL

TRYSAN RESEARCH, INC.
1190 SPRING CREEK PLACE, SPRINGVILLE, UT. 84663

IF THERE ARE ANY PROBLEMS ASSOCIATED WITH THIS FAX TRANSMISSION, PLEASE CONTACT US AT FAX # 801 489-8487, OR TELEPHONE # 801 489-8444.

TO: DESIGN NUTRITIONAL PRODUCTS Attn: Wes

FAX: 226-8496

FROM: Calvin McCausland

DATE: 2 Sep 93

PAGES: 1, including this page.

SUBJECT: P.O. for I3C and Diindolymethane -**CORRECTED.**

Re: P.O. 2533

I make an error in the calculations for the above P.O. dated 2 Sep 92. Please discard the previous fax and use this corrected sheet.

We wish to place an order for the following:

QUANTITY	ITEM	PRICE/KG	TOTAL
2.4 Kg	I3C	[redacted]	[redacted]
0.5 kg	Dindolymethane	[redacted]	[redacted]

The above is to be combined with **72 kg** of broccoli powder that we will supply to produce a 4% concentrate in indoles.

Note that this is a separate P.O. from that of yesterday (P.O. 2532) for a Nutrin-3 mix and Glycogen.

Have you acquired any type of mixer where in we could get from you a uniform blend for this and the other mix?

21 Sep 93 Note: Correct to 58 lbs (26.4 kg) broccoli powder which is a 10% indole concentrate.

50# 13#

DESIGN NUTRITIONAL PRODUCTS

Nutripro 21 Sep 93 Page 10

Lot 3

60 Tabs/Unit

ITEM	MG PER TABLET	KG PER M UNITS	KG PER 4M UNITS	LBS PER 4M UNITS
Broccoli powder	110	6.600	26.400	58.1
I3C	10	0.600	2.400	5.3
Diindoylmethane	2	0.120	0.500	1.1
Broccoli powder	190	11.400	45.600	100.0
	312	18.720	74.900	164.5

MANUFACTURING BATCH RECORD

PRODUCT NAME: NutrPro Tablets, 1080 mg LOT NUMBER: —

ORDER NUMBER: 2533 ORDER QUANTITY: 4000 Bottles

DATE: 25 Oct 93 FINISH: BOTTLES, 60 COUNT

MATERIAL SUPPLY

General: The quantities of raw materials issued will be the discrete amounts shown on the Manufacturing Batch Rec

1. rify cleanliness of weighing area and equipment to be used A. C
2. Locate and stage the raw materials to be used.
3. Record the Lot number.
4. Visually inspect each raw material for physical stability and for any contamination with foreign matter. Report exceptions to quality assurance, and obtain the decision of QA.
5. Take the properly labeled container to the weighing area for staging.
6. Weigh each item on the scales into a plastic lined container and record.
7. Label each container with its contents, lot number, weight, and the batch lot number.
8. If you use the same raw material from more than one lot record the different raw material Lot numbers record the amounts from each.
9. Repeat the previous steps until completed.

RAW MATERIALS REQUIRED FOR THIS LOT NUMBER

Ingredient	Quantity Required (Kg)	Raw Material Lot No.	Quantity Supplied (Kg)
Broccoli Powder Conc. 10%	29.5	4ABT	29.5
Total	29.5	"	29.5

Total weight of material supplied: 29.5 kg

Number of containers: 1

Completed by: A. C Date: 10/26/93

Designed Nutritional Products

145 North Geneva Road, Vineyard, Utah 84057 phone (801)224-4518 fax (801)226-8496

CERTIFICATE OF ANALYSIS

3,3'bis-indolylmethane

lot #4ADJ

Appearance:	Light tan, crystalline powder
FTIR Spectra:	Conforms to standard
Purity (by HPLC):	91%
Melting Point:	148-151°C

Nancy K Edge

Nancy K. Edge
Analyst

2/7/94

Designed Nutritional Products
145 North Geneva Road, Vineyard, Utah 84058 Phone (801)224-4518 Fax (801)226-8496

CERTIFICATE OF MANUFACTURE

25% INDOLES MIXTURE

This product contains 25% dietary indoles by content and is certified by DNP to meet specifications as set by DNP.

Indole-3-carbinol batch #s: PBB20AGP01-1500, PBB28AGP01, PBB29AGP01
Bis(3-indolyl)methane batch # PJA18AGP01-1599
Broccoli Powder lot # 1043D
Ascorbigen batch # 4ACH-BDE

25% Indoles batch # PGB25MJK02

LOT # 01054

Certified by: _____ Date: 11-3-95
Mark Karamesines
Production Manager

Reviewed by: _____ Date: 11-3-95
Dean D. Evans
Director of Laboratory Services

Appendix II

This appendix contains the protocol used to survey patients with Recurrent Respiratory Papillomatosis (RRP), revised charts of the data with clarified column headings, separately showing DIM and I3C use as dietary supplements, and a summary of these survey findings.

1. Protocol for Survey

2. Data and Results

3. Conclusions

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Appendix II.
BioResponse Survey of Individuals with Recurrent Respiratory Papillomatosis (RRP) and Long Term Experience with I3C or DIM Supplementation

I. Survey Protocol

A. Survey Participant Selection: An announcement for participants to take part in a telephone survey concerning I3C and DIM supplementation was circulated by E-mail to all members of the RRP Foundation accessible by this means.

1. Respondents indicating the longest term use of I3C or DIM were contacted by telephone.

II. Structure of the Telephone Interview:

A. The following questions were asked of all participants:

1. Age, Weight and Sex.
2. Duration in years of RRP symptoms.
3. Duration of I3C or DIM use in consecutive months.
4. Side effects noted: as an open question.
5. Presence or absence of particular side effects was asked including: dizziness, unsteadiness of gait, nausea, vomiting or diarrhea had been noted.
6. I3C or DIM dose in mg per person per day.
7. Whether the individual had participated in the Rosen study.

B. The following question was asked of the last 4 participants.

1. Was a physician involved in the supervision of I3C or DIM supplementation?

III. Notes on presentation of tabulated responses:

A. In cases where a period of supplementation with I3C was followed by a period of supplementation with DIM, a single individual was listed both in the I3C and the DIM table

B. No individuals were identified whose results had already been described in the Rosen study.

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LONG TERM INDOLE-3-CARBIONOL (I3C) USE IN HUMANS
Survey of Individuals with Recurrent Respiratory Papillomatosis (RRP)

Initials	Weight Kg	Sex	RRP yrs	Age yrs	I3C Use in months	Side Effects	I3C Dose mg/day	I3C Dose mg/kg/day	Physician Supervision I3C use
Ariel	18.6	F	5.8	6	32	none	400	21.5	
M.W	19	F	2.5	5	30	none	300	15	yes
Jonathan D	20.9	M	3.5	8	24	none	300	14.3	
Christine S	53.6	F	14	41	36	none	400	7.5	
Jeff N.	84	M	5	50	48	none	600	7.1	
Linda C.	60	F	7	42	34	none	400	6.6	
Emily S.	30.5	F	8	10	40	none	200	6.5	
M.B.	84	M	4	45	18	none	500	5.9	yes
Michael G	77.2	M	20	53	52	none	35	5.8	
Ralph S.	70	M	5	71	34	none	400	5.7	
Ron B.	75	M	8	41	9	none	400	5.3	
Ed L.	79.5	M	8	38	6	none	400	5	

LONG TERM DIINDOLYLMETHANE (DIM) USE IN HUMANS
Survey of Individuals with Recurrent Respiratory Papillomatosis (RRP)

Initials	Weight Kg	Sex	RRP yrs	Age yrs	DIM Use in months	Side Effects	DIM Dose mg/day	DIM Dose mg/kg/day	Physician supervision of DIM use
Jonathan D	20.9	M	3.5	8	12	none	300	14.3	
G.W.	26.3	M	5	6.5	16	none	300	13.2	yes
Ron B.	75	M	8	41	4	none	400	5.3	
Chris N.	70	M	5.5	30	13	none	400	5.7	
Ed L.	79.5	M	8	38	36	none	400	5	
M.R.	70	M	2	35	8	none	300	4.2	yes

Appendix II.
BioResponse Survey of Individuals with Recurrent Respiratory Papillomatosis (RRP) and Long Term Experience with I3C or DIM Supplementation

IV. Survey Conclusions:

A. Three individuals reported taking an I3C dose in the elevated range of 14-21 mg/kg/day for 2-2.5 years without side effects. These individual were all children under 10 years of age. Tolerance of these doses was in excess of the tolerated dose of 6 mg per kg reported by Rosen in adults (See Appendix 2 in the Additional Safety Information submitted May 18, 1998)

B. DIM supplementation at a dose of 13-14 mg per kg was reported by two individuals for 16 and 12 months respectively.

C. No side effects were reported or elicited with questioning. A single individual (M.R.) reported improved symptoms of constipation on 4.2 mg/kg/day of DIM. Various individuals reported mild gastric upset with I3C which improved when taken with food.

D. This survey provides limited evidence of tolerance of DIM at more than 10 times the maximal proposed supplement use of DIM at 1 mg/kg/day.

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Appendix III

Summary of Physician Reports Concerning Supervised Use of I3C as a Dietary Supplement.

Each summary includes number of patients supervised, dose of I3C in use, and responses to questions concerning side effects.

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Appendix III.
Physician Supervised Use of Supplemental Indole-3-Carbinol (I3C)

Two physicians with known experience in caring for RRP patients on I3C supplementation were contacted by telephone and asked to comment on the following points:

- Number of patients on I3C supplementation at 6mg/kg/day now being clinically monitored:
- Reports of side effects in these or other patients.
- Experience with dosage in excess of 6 mg/kg/day of I3C
- Longest duration of supplementation with I3C at 6 mg/kg/day

I. Harry Hoffman, M.D.
University of Iowa
Department of Otolaryngology
1. Number of patients on I3C: 15
2. Side Effects: None
3. Experience with higher dose: none.
4. Longest duration of supervised treatment: 1 year.

II. Clark Rosen, M.D.
Department of Otolaryngology
University of Pittsburgh
1. Number of patients on I3C: 100
2. Side Effects: None
3. Experience with higher dose: none.
4. Longest duration of supervised treatment: 3.5 years.

IV: Final Notes: This physician reported experience adds 115 medically supervised individuals on I3C supplementation to those previously reported in published literature. The patients reported in the present survey by Dr. Rosen were separate from the 18 individuals reported in the Rosen paper and included in Appendix 2. of the May 18, 1998 new safety information on DIM

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Appendix IV

Inhibition of Cytochrome (CYP) Enzyme Induction and CYP Enzyme Activity by Diindolylmethane (DIM)

DIM inhibits activation of the Aryl Hydrocarbon Receptor (AhR) and is a CYP1A enzyme inhibitor

This appendix contains a summary of the inhibitory actions of DIM as presented by David Williams, Ph.D. at the May 29, 1998 meeting. Also included are references to peer reviewed articles and Ph.D. dissertation theses on file, establishing these actions of DIM. In addition, reference to experimental work showing that DIM does not cross the placenta is provided.

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Appendix IV
Inhibitory Activity of DIM towards CYP Enzymes and the AhR.

BioResponse presented experimental and published data supporting the role of DIM as an inhibitor of the Aryl Hydrocarbon Receptor (AhR) at the levels proposed for use. Dr. David Williams, an authority on I3C and DIM responses after ingestion by mice and rats, presented published data showing inhibition of CYP enzyme in the relevant tissue concentration range encountered in vivo in animals.

Dr. Williams, affirmed "that results todate in vivo confirm that DIM is a weaker inducer than I3C. We estimated an ED_{50} in mammalian liver for CYP1A1 by DIM of 64 uM. Recent work in vitro with high precision liver slices from the rat yielded an ED_{50} of 50uM (1,2), which agrees very well with the previous estimate. We have additional data documenting that DIM is a potent inhibitor of both rat and human CYP1A1, as well as human CYP1A2 with a K_M in the range of 10 uM (3). Following dosing of rats with 147 mg/kg/day for 7 days with I3C, the liver concentration of DIM was only 3-6 uM (4). Taken together, these data predict that even at relatively high doses, the inhibitory effect of DIM would predominate over induction. That is, even if there were a small amount of CYP1A1 protein induced, it would be rendered catalytically inactive".

Additional data regarding the placental transfer of I3C condensation products were also presented by Dr. Williams. Following treatment of pregnant rats with I3C, livers from the newborns were analyzed by HPLC and Mass Spectrometry. The presence of the linear trimer condensation product from I3C was demonstrated. DIM and its hydroxylated metabolite were notably absent. These results are important in a safety assessment since neither DIM nor its metabolite were present in fetal or neonatal livers of rat offspring. This work, demonstrating lack of placental transfer of DIM is on file as part of a Ph.D. dissertation thesis (5), as well as appearing in an upcoming publication (6)

References:

1.Oganesian, A. et al, submitted to Chemical -Biological Interactions, 1998.

2.Oganesian, A., 1997, Ph.D. Thesis, Oregon State University, on file O.S.U., Department of Nutritional Science

3.Stresser et al, 1995, J. of Biochemical Toxicology 10:191

4.Stresser et al, Drug Metabolism and Disposition, 1995, 23:965

5. Larsen-Su, S., 1998, Ph.D. Thesis, Oregon State University, on file O.S.U., Department of Nutritional Science.

6.Oganesian, A, Williams, D., et al, submitted to Cancer Letters, 1998.

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