



DEPARTMENT OF HEALTH & HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

Public Health Service

Memorandum

Date . APR - 9 1998 3 8 9 8 '98 APR 14 P 2 :27

From Senior Regulatory Scientist, Regulatory Branch, Division of Programs & Enforcement Policy (DPEP), Office of Special Nutritionals, HFS-456

Subject 75-day Premarket Notification for New Dietary Ingredient

To Dockets Management Branch, HFA-305

New Dietary Ingredient: 3,3'-diindolylmethane

Firm: BioResponse, L.L.C.

Date Received by FDA: August 19, 1997

90-day Date: November 1, 1997

In accordance with the requirements of section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification for the aforementioned new dietary ingredient should be placed on public display in docket number 95S-0316 after November 1, 1997.

Robert J. Moore, Ph.D.

95S-0316

RPT 18



3 8 9 9 '98 APR 14 P 2 :27

APR - 9 1998

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

Dear Mr. Zeligs:

This is in response to your letter to the Food and Drug Administration (FDA) dated August 11, 1997, making a submission pursuant to 21 U.S.C. 350b (section 413 of the Federal Food, Drug, and Cosmetic Act (the Act)) for a new dietary ingredient. Your letter notified FDA of your intent to market a dietary supplement containing a new dietary ingredient, diindolylmethane (DIM).

21 U.S.C. 350b(a)(2) requires that a manufacturer or distributor of a dietary supplement that contains a new dietary ingredient submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information which is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. Under section 350b(a)(2), there must be a history of use or other evidence of safety establishing that the new dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If these requirements are not met, the dietary supplement is deemed to be adulterated under 21 U.S.C. 343(f)(1)(B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

FDA has carefully considered the information in your submission, and the agency has significant concerns about the evidence on which you rely to support your conclusion that a dietary supplement containing DIM will reasonably be expected to be safe. Your submission states that the recommended daily dietary exposure to DIM will be 50-200 milligrams (mg)/day in women and 50-250 mg/day in men (approximately 3.5 mg/kilogram body weight (kg bw)/day). In your submission, you cite four lines of evidence purporting to establish that DIM may be safely used as a dietary supplement in humans. However, FDA believes that you have not considered significant limitations of the evidence you cite and findings in the studies cited that raise unresolved, serious potential health risks associated with the amounts of DIM you propose for your products.

1. Normal Dietary exposure.

You cite as evidence of safety that DIM is a normal constituent of the diet, derived primarily from precursors found in cruciferous vegetables. However, you provide no data on, nor an estimate of, normal dietary exposure to DIM itself. DIM is one of a number of indole metabolites (others include indole-3-carbinol and indole-3-acetonitrile) that result from an enzyme-mediated autolytic action on certain glucosinolate precursors, such as glucobrassicin, present in cruciferous vegetables. Using data provided in your submission, we estimated an upper percentile dietary exposure to DIM, as a constituent of the diet, to be approximately 0.32 mg/kg bw/day. Consideration of other additional factors, such as the inefficient conversion of glucobrassicin to DIM, suggests that the actual dietary DIM intake is most likely even below this estimated level of exposure. Thus, the estimated dietary exposure to DIM is at least an order of magnitude lower than the exposure that would result from the proposed use of your product. Therefore, dietary exposure to DIM is not adequate to support the safety of your proposed dosage.

2. Short-term studies of DIM exposure in animals.

You cite three studies that investigated the effects in rats of acute oral exposure to DIM at levels from 1.5 to 58 mg/kg bw/day. FDA does not agree that short-term exposure studies in animals adequately reflect effects of long-term dietary exposure in humans. Moreover, contrary to your assertion that these studies demonstrate that DIM did not result in adverse effects in the experimental animals, these studies provide evidence that raise significant concerns about the safety of DIM.

First, in one study,¹ DIM was administered in conjunction with exposure to a chemical carcinogen. Such an experimental design is not adequate to develop evidence that exposure to DIM itself is safe because it does not parallel the physiological status of humans using DIM or the conditions for the development of cancer in humans.

In the second animal study you cited,² rats fed a diet that minimally induced the mixed-function oxidase (MFO) system (i.e., "minimal inducing diet") were also exposed to oral DIM and an induction of hepatic MFO was observed. However, this experimental paradigm is not analogous to the human situation, in that it does not reflect exposure to both dietary and pharmacological agents that humans also encounter and that may also influence induction of MFO activity. Thus, it is questionable whether data obtained from experimental animals being fed a minimal inducing diet is valid for use in a safety assessment of DIM exposure in humans. It would not adequately address the many factors, and their possible

¹Wattenberg LW, Loub WD. *Cancer Res* 1978; 38:1410-13.

²McDanell R, McLean AEM, Hanley AB, et al. *Fd Chem Toxic* 1987; 25:363-8.

interactions, that affect the MFO system in humans. This is a potentially relevant issue because the induction of MFO activity is able to both activate and inactivate chemicals, including carcinogens.

In the third animal study you cited,³ the data demonstrated that a single oral exposure to DIM induced hepatic estrogen-2-hydroxylase activity. However, this study also showed that DIM exposure induced cytochrome P450 isozymes through a mechanism involving the aryl hydrocarbon hydroxylase (Ah) receptor, which is the receptor at which 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) acts. Your submission does not address the issue of potential deleterious effects associated with the stimulation of the Ah receptor or related cytochrome P450 isozymes that have been noted by others (for example, see Guengerich FP, *Am Scientist* 1993;440-7).

These three studies do not provide adequate information to perform a safety assessment for long-term DIM exposure in humans, and more importantly, they identify potential hazards associated with long-term dietary exposure to elevated intakes of DIM.

3. Exposure to indole-3-carbinol as a model for exposure to DIM

The third line of evidence that you cite as a basis for concluding that DIM is safe are studies of animal and human exposure to indole-3-carbinol (I3C). You argue that this line of evidence is valid because of evidence for the conversion of I3C to DIM in the gastrointestinal tract. The animal studies you cite provide evidence that you assert supports the safety of orally administered I3C, and by extension, DIM. However, your consideration of the available scientific evidence seems to ignore findings that suggest risks associated with oral exposure to I3C. For example, Kim et al.⁴ demonstrated that I3C enhanced liver and thyroid neoplastic development in rats when given subsequent to exposure to chemical carcinogens. These authors cited other studies that also have found that when animals are exposed to I3C subsequent to the administration of carcinogenic agents, tumor production is increased.⁵

Another problem with your safety assessment is that your calculation of the allowable daily intake (ADI) of I3C does not follow generally accepted scientific principles. To arrive at an estimate of an ADI for I3C (or DIM) based on animal work, a traditional safety assessment would require identifying the most sensitive toxicological endpoint and making an

³Jellinck PH, Forkert PH, Riddick DS, et al. *Biochem Pharmac* 1993; 45:1129-36.

⁴Kim DJ, Han BS, Ahn B et al. *Carcinogenesis* 1997; 18:337-81.

⁵Wilker C, Johnson L, Safe S. *Tox Appl Pharm* 1996; 41:68-75; Shertzer HG, Sainsbury M. *Fd Chem Toxic* 1991; 29:237-42; Grubbs CJ, Steele VE, Casebolt T et al. *Anticancer Res* 1995; 15:709-16; Bradlow HL, Michnovicz JJ, Teland et al. *Carcinogenesis* 1994; 12:1571-4.

adjustment for extrapolation from animals to humans. With respect to the data you included in your submission, the most sensitive toxicological endpoint for I3C appears to be the male reproductive system (see study by Grubbs et al., footnote 5). Abnormalities in the reproductive system were seen in male offspring of pregnant rats exposed to a single oral dose of 1 mg I3C/kg bw on day 15 of gestation. This assessment could be based on the lowest observed adverse effect level (LOAEL) of 1 mg I3C/kg bw, or 0.79 mg DIM/kg bw and an uncertainty factor of at least 100 (10 for intra species variability x 10 for interspecies variability; an additional factor for derivation from LOAEL instead of a no observed adverse effect level (NOAEL) could also be included). Thus, the resulting acceptable daily intake for DIM would be 0.008 mg/kg bw, which is approximately three orders of magnitude less than the maximum dosage of DIM you recommended for your product.

We are concerned that in evaluating the effects of I3C in animals, you did not undertake recognized safety assessment procedures to perform a safety evaluation. You make direct comparisons between the doses of exposure seen in animals to the dose you intend to use in humans without making any adjustments for the extrapolation of animal data to humans. You argue that doses of DIM or I3C associated with toxic effects in animals are well above your recommended intakes of DIM; however, you fail to provide an adequate basis for using margins of safety that are less than generally recognized as appropriate.⁶ For example, you rely on margins of safety of 29X, 38X, or 80X, when the standard safety/uncertainty factor for this type of extrapolation is 100X.

4. Human exposure to I3C.

Your submission cites two studies in humans as evidence that DIM is safe. We disagree that these studies support the safety of long-term exposure to DIM. Neither study was longer than 3 months in duration, which precludes using either one as a basis for concluding that your proposed dosage of DIM is safe for long-term use.

In summary, the information in your submission does not constitute “a history of use or other evidence of safety establishing that the new dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe” under 21 U.S.C. 350b because: (1) the proposed recommended dietary supplement dosages far exceed the amount consumed in a typical diet; (2) the data from the animal studies cited in your submission are inadequate to perform a

⁶WHO, Principles for the safety assessment of food additives and contaminants in food. Environmental Health Criteria 70: WHO, Geneva, 1987; IRIS, Background documents: Reference Dose (RfD): description and use in health risk assessments. US EPA, Integrated Risk Information System, 1993; ATSDR, Interpretation of minimal risk levels, from toxicological profile for acetone, Appendix A. US DHHS, PHS, Agency for Toxic Substances and Disease Registry, TP-93/01, 1994.

Page 5 - Mr. Michael A. Zeligs

safety assessment for DIM used as a dietary supplement in humans; (3) your safety assessment relied on studies that included paradigms involving the administration of exogenous carcinogens to assess the safety or toxicity of DIM alone; (4) your safety assessment compared DIM or I3C exposures and their effects in animals to dosages in humans without taking into account uncertainties associated with animal to human extrapolations; and (5) the significance of toxic responses seen in some animal studies coincident with exposure to I3C was not recognized or reasonably considered.

Based on the information in your submission and other relevant scientific information, FDA disagrees with your conclusion that a dietary supplement containing DIM will reasonably be expected to be safe. Therefore, your product is adulterated under 21 U.S.C. 343(f)(1)(B) because it contains a new dietary ingredient for which there is inadequate information to provide reasonable assurance that such ingredient does not present a significant or unreasonable risk of illness or injury.

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

cc:

HFA-224 (w/incoming)

HFA-305 (Docket No. 95S-0316)

HFS-22 (CCO)

HFS-308 (Bolger, Assimon)

HFS456 (r/f, File)

HFS-450 (r/f, File)

r/d:HFS456:RMoore:3/20/98

revised per GCF-1:LNickerson:3/27/98

revised per HFS-308:SAssimon:4/6/98

Init:GCF-1:LNickerson:4/7/98

f/t:rjm:HFS-456:4/7/98:docname:dim.osn:disc27

MEMORADUM OF TELEPHONE CONVERSATION

3900 '98 APR 14 P2:27

August 29, 1997, 12:37 p.m.

Between: Ms. Elizabeth Zeligs
BioResponse, L.L.C.
P.O. Box 288
Boulder, Colorado 80306-0288

and: Jeanne E. Latham, R.D., M.S., L.D.
Consumer Safety Officer
Regulatory Branch
Division of Programs and
Enforcement Policy
Center for Food Safety and
Applied Nutrition



Subject: Reference number 22 from the 75 Day Notification of a New Dietary Ingredient

I called to speak with Michael A. Zeligs of BioResponse, L.L.C. Elizabeth Zeligs said that he was unavailable. I explained to Ms. Zeligs that FDA has received the notification and that reference number 22 appeared to be missing. I indicated that we would need it for the packet to be complete, and that once we received it, depending upon whether it was substantive, the 75 days may need to be changed. She stated that she would need to speak with Dr. Zeligs and would call me back within a half hour. I said that that would be fine.

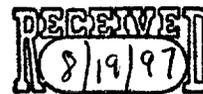
At 1:00 p.m. Dr. Zeligs returned my call. He stated that there was no formal report or paper work for reference number 22 for the 75 Day Notification. Rather this "reference" refers to an anecdotal "report (account) based on personal use by William Stern." He stated that he did not mean for this to be confusing, but wanted it to be part of the submission. He said that he would be available to answer any other questions we had.

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

August 8, 1997

3901 '98 APR 14 P2:27

Elizabeth A. Yetley, Ph.D.
Director
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. 20204



Dear Dr. Yetley:

Pursuant to Section 8 of the Dietary Health and Supplementation Act, BioResponse is pleased to submit the attached New Dietary Ingredient Dossier for Diindolymethane (DIM).

This document is the basis upon which BioResponse has concluded that DIM, when used as a dietary ingredient in dietary supplements, will reasonably be expected to be safe.

BioResponse regards the entire information provided as confidential business information. BioResponse therefore requests that the Secretary continue to hold the entire New Dietary Ingredient Dossier for DIM confidential past the usual 90 day non-disclosure date.

Your attention to this matter, and efforts in our behalf by members of your staff are greatly appreciated.

Respectfully Submitted,

A handwritten signature in black ink that reads "Michael A. Zeligs". The signature is fluid and cursive.

Michael A. Zeligs
BioResponse, LLC

54265

Telephone 303-4473841

Facsimile 303-938-8003

E-mail zeligsmd@sni.net

**PREMARKET NOTIFICATION FOR A NEW DIETARY
INGREDIENT**

I. DATE: August 11, 1997

II. MANUFACTURED BY: BioResponse, L.L.C.
P.O. Box 288
Boulder, Colorado
80306-0288

III. DIETARY INGREDIENT NAME:

3,3'-Diindolylmethane (DIM)

IV. DESCRIPTION: 3,3'-Diindolylmethane (DIM) is a natural compound found as a component of cruciferous vegetables. These include cabbage, cauliflower, Brussels sprouts, turnips, radish, and Chinese cabbage (hakusai). DIM is now newly available as a pure, crystalline material for addition to the diet. BioResponse has developed DIM for use as a dietary ingredient by processing the crystalline form for improved gastrointestinal absorption and increased shelf life. Processing includes reduction of DIM particle size and admixture with absorption enhancing food grade materials. Substances added during processing may include Vitamin E Succinate, Phosphatidylcholine, and food-grade starch. The proprietary processing steps include microparticle formation and microencapsulation, or coating of DIM microparticles, to form a composite powder. The composite powder can be mixed with excipients such as silica, as well as with other established dietary supplement ingredients. Protection from light is provided by opaque gelatin capsules. The standard dosage form will contain 50 milligrams (mg) of Diindolylmethane in each opaque capsule.

V. RECOMMENDED CONDITIONS FOR USE:

A. Dosage and Label Instructions

The recommended daily dose of BioResponse's Diindolylmethane is from 50 to 200 milligrams (mg) per day of Diindolylmethane for women and 50 to 250 mgs per day for men.

This corresponds to a maximum dose of approximately 3.5 milligrams per kilogram (kg) of body weight. Capsules containing BioResponse's Diindolylmethane are to be taken orally as a dietary supplement. The supplement is to be taken with fluids, preferably at times during which the stomach is otherwise empty.

BioResponse's Diindolylmethane is to be used by children or women who are pregnant, nursing, or capable of becoming pregnant only under the supervision of a health-care professional. BioResponse's Diindolylmethane is not recommended for use by women taking oral contraceptives (birth control pills).

Each bottle will contain instructions for use as follows:

Directions: Take one to five capsules daily, with fluids at a time when the stomach is empty. Women should not exceed four capsules per day. Men should not exceed five capsules per day.

Warnings: Children or women who are pregnant, nursing, capable of becoming pregnant, or already using oral contraceptives (birth control pills) should use this product only under the supervision of a health care professional. Persons taking prescription medications should consult with a health-care professional before using this product. Do not exceed the recommended dosage.

B. Background of labeling recommendations for safety assurances.

Centuries of human consumption of DIM containing cruciferous vegetables and scientific investigation have provided the background for human use of DIM as a dietary supplement. Since pure DIM is now available for the first time, BioResponse has developed a new dosage form in order to provide a convenient and safe way for individuals to increase their daily intake of DIM. The usage guidelines, outlined above, address the safest manner of use.

In addition to adding to the cruciferous vegetable component of the diet, the purpose of DIM supplementation is to promote safe estrogen status through dietary support of the 2-hydroxy pathway of estrogen metabolism. It is reasonable that altered estrogen activity may be unwanted in the situations of pregnancy and oral

contraceptive use. Label warnings provide for physician controlled use in these settings.

Though DIM supplementation has not been formally studied in human pregnancy, there is related evidence of safety in animal reproduction.

In a separate study of indole-3-carbinol [2], subtle reproductive effects were suggested to occur in male offspring of female rats exposed to indole-3-carbinol during pregnancy. This study found differences in sperm maturation rates in the male offspring from these pregnancies. The suggestion was made that effects on sperm maturation in male offspring can be induced by exposure of the male fetus in utero to maternal, dietary indole-3-carbinol [2]. However, the small sample size and inconsistencies in the data make the adequacy of statistical power and conclusions regarding indole-3-carbinol doubtful. As presented in the following section, VI., F., subsection ii., the greater stability of pure DIM over indole-3-carbinol following gastric digestion will add to its safety in reproductive settings.

Recommendations for use specify a dosage range limiting maximal dosage to approximately 3.5 mg per kg per day. As will be seen in the following section, VI. "Evidence for Safety of BioResponse's Diindolylmethane (DIM)", extended use of the DIM precursor, indole-3-carbinol in animals at high dosage for periods of 8 months to 2 years proved safe. Dosage in excess of 100 times the 3.5 mg per kg maximal dose recommended for DIM was shown to be safe in these studies [3-5].

In other studies at high dosage of indole-3-carbinol, when animals were first pre-treated with experimental carcinogens, and subsequently begun on high dose indole-3-carbinol, increases in carcinogenicity and tumor promotion have been reported. An example of such work, summarizing these observations, is the report

by Kim et al. [6]. This study showed enhancement of induced liver and thyroid gland neoplasia over carcinogens alone when indole-3-carbinol use was initiated at 343 mg per kg following exposure of rats to multiple carcinogens. In this and in other studies like it, referenced by Kim [6], the findings of tumor promotion are invariably seen following high dosage of carcinogens not relevant to human exposure. The pharmacologic doses of indole-3-carbinol used are not comparable to human dietary and supplemental intakes. Further investigation of this effect has revealed inconsistent observations in different species and loss of the promotional effect at low doses of indole-3-carbinol.

The recommended maximal dosage of 3.5 mg per kg of DIM is approximately 100 times less than in experiments showing tumor promotion in carcinogen-primed animals treated with indole-3-carbinol.

VI. EVIDENCE FOR SAFETY OF BIORESPONSE'S DIINDOLYLMETHANE (DIM)

A. DIM is a normal component of the human diet.

In nature, DIM occurs in association with cruciferous vegetables. DIM, therefore, has been present in the human diet since ancient times in proportion to the quantity of cruciferous vegetables consumed. DIM arises from glucobrassicin, a precursor glucosinolate phytochemical, via the release and spontaneous combination of indole-3-carbinol molecules. Normal intake of glucosinolates can be significant, with levels of glucobrassicin reported to be as high as 672 mgs per 100 grams in some crops such as Savoy Cabbage [7]. On a

weekly basis, normal glucobrassicin intake can total thousands of mgs contributed by various cruciferous vegetables, since per capita consumption totals hundreds of grams per week. The following Table documents these quantities and emphasizes the greater amounts of cruciferous vegetables consumed in Asian societies:

Table 6
PER CAPITA CONSUMPTION OF SOME
BRASSICA VEGETABLES IN THE U.K.
(1978), U.S. (1978), AND JAPAN (1975)

Vegetable	g/person/week
U.K.¹⁷²	
Cabbage	137.2
Cauliflower	78.3
Brussels sprouts	62.4
Turnip/swede	38.6
U.S.¹⁶⁷	
Cabbage	77.0
	11.2 (sauerkraut)
Cauliflower	9.8
	4.2 (frozen)
Brussels sprouts	2.1 (frozen)
Broccoli	11.2
	11.9 (frozen)
Japan¹⁷³	
Radish (daikon)	232.4
Cabbage	136.5
Chinese cabbage (hakusai)	159.6
Salt fermented leafy vegetables	135.1
Salt fermented root vegetables	125.3

Japanese data from *Present Status of National Nutrition in Japan*, Ministry of Health and Welfare, Daiichi Shuppan, Tokyo, 1975.

Table from: Fenwick, G.R., Heaney, R.K., and Mullin, W.J., "Glucosinolates and Their Breakdown Products in Food and Food Plants", *CRC Crit. Rev. Food Sci. Nutr.*, 1983, Volume 18, Issue 2, page 141.

During preparation or chewing of cruciferous vegetables, release of the sequestered plant enzyme, thioglucosidase, triggers an autolytic process producing a series of three-substituted indoles. Among these are indole-3-carbinol, indole-3-acetonitrile, and ascorbigen which is a combined molecule of indole-3-carbinol and ascorbic acid. Indole-3-carbinol and ascorbigen subsequently combine with themselves to form the double molecule, 3,3'-diindolymethane (DIM). (See Figure 1. in Bradfield and Bjeldanes [8]). These authors demonstrated the presence of DIM in extracts of blended cruciferous vegetables. Naturally produced DIM is abbreviated as I33' in this report (See Figure 4 in reference [8]). DIM levels in food are greatly increased after passage of cruciferous vegetables through the acidic environment of the stomach due to condensation of more plentiful indole-3-carbinol into DIM.

Based on epidemiologic research, the intake of cruciferous vegetables is widely regarded as important and beneficial. The use of DIM as a cruciferous indole supplement offers advantages over simply increasing dietary cruciferous vegetable intake for the following reasons: (1) Increasing cruciferous vegetable intake to levels traditionally eaten by rural Chinese in low cancer regions can be associated with the minor complaints of dyspepsia and flatulence. (2) Increased cruciferous vegetable intake will also increase exposure to isothiocyanates and other glucobrassicin breakdown products of uncertain benefit[9]. (3) Individual differences in digestive function make the intra-gastric formation of substances of proven benefit, like DIM, subject to wide individual variation. (4) With human aging, low gastric acidity diminishes the gastric production of DIM from its inactive precursors and limits DIM's availability from cruciferous vegetable sources.

The availability of pure DIM now permits low-dose dietary supplementation to add to indoles available from dietary vegetables. The work of Michnovicz and Bradlow [10] specifically identified dietary indoles, like DIM, as capable of inducing Cytochrome P450 enzymes beneficial in the control of estrogen metabolism. DIM is the most active dietary indole in promoting enhanced production of 2-hydroxyestrone. Increased 2-hydroxy estrone supports a healthy

hormonal balance and stimulates the natural metabolic pathways for estrogen in men and women.

B. History of supplemental use of DIM.

DIM was first used as a supplement in 1978 by Wattenberg and Loub [11]. These investigators used a dose of approximately 100 mgs per kg of body weight of DIM in rats and showed prevention of benzopyrene induced gastric cancer. The DIM was administered mixed with the rat's food for periods of one and two months. Cancer was statistically reduced, and in both the one- and two-month groups there was normal weight gain compared to control. Even more surprisingly, a single oral dose of 75 milligrams per kg of DIM was shown to prevent mammary cancer induced by dimethylbenzanthracene[11].

In 1987, McDanell et al.[7] compared the addition of dried cabbage and purified indoles (DIM, and indole-3-carbinol) to a minimal inducing diet. They thus observed the inducing effect of dietary indoles on mixed function oxidase enzymes in the liver, and small and large intestines of rats. These animals were safely fed DIM for a period of 5 days. Assuming a daily food intake of 10 grams per rat and an average weight of 200 grams per animal, the dose of DIM given was approximately 10 mgs per kg per day. This dose was adequate to produce a fourfold induction of mixed function oxidase in the livers of DIM-fed rats and was comparable to consuming a 25% cabbage diet. There was no change in liver weight. Interestingly, DIM had activity only in the liver which spared the large intestine, indicating more efficient absorption compared to indole-3-carbinol (See Table 5 of reference [7]).

Recently, the activity in the proposed dosage range of pure DIM (0.5 to 3.5 mgs per kg) for humans was demonstrated to produce the desired shift of estrogen metabolism in rats. Following a single oral dose, Jellinck et al. [12] demonstrated that 5 mgs per kg of DIM (1 mg per 200 gram rat) was sufficient to produce an enhanced 2-hydroxylase pathway of estrogen metabolism.

Recognition of the importance of dietary indoles in health promotion lead first to the manufacture of "mixed indoles". This

mixture consisting of DIM and an array of other indole substances has been in use as a dietary ingredient for human use. Though predominantly DIM, "mixed indoles" contain an array of indole compounds not necessarily found in the human diet. New manufacturing techniques have provided pure DIM allowing BioReponse's DIM preparation to be formulated.

Newly developed, pure, DIM, precisely resembles DIM present in the human diet. However, in its pure crystalline form this new dietary ingredient is more homogeneous and concentrated than DIM found in food. Processing pure DIM by BioResponse into microparticles and the coating of these tiny particles with lipid compatible substances like Vitamin E Succinate and phosphatidylcholine produces a new form of DIM not found in nature. This new dietary ingredient form of DIM is readily absorbable in the human digestive tract.

C. Ingestion of indole-3-carbinol results in the intra-gastric formation of DIM.

Since 1990, the availability of synthetically pure indole-3-carbinol has led to its use as a dietary supplement. However, continued investigation of indole-3-carbinol's in vivo activity indicated that indole-3-carbinol lacked biologic activity without first passing through the acidic environment of the stomach. This fact was best demonstrated by Dashwood et al. [13], who bypassed activity of the stomach by injecting dietary indoles directly into developing trout embryos. Following injection into the yolk sack indole-3-carbinol was ineffective at preventing aflatoxin-induced tumors while DIM was effective. Other investigators fed indole-3-carbinol to rats at a dose of 30 mgs per kg, removed the stomach contents following digestion, and analyzed the indoles present. They found that 3.5% of the dose was converted to DIM during transit through the stomach [14]. Thus, gastric digestion naturally augments the intake of DIM, present in precursor form in diets rich in cruciferous vegetables. This knowledge allows the use of toxicologic research on indole-3-carbinol to support the safety of DIM as long as the ingested indole-3-carbinol has passed through an acidic gastric environment.

D. The safety of DIM is supported by animal toxicology studies of its precursor, indole-3-carbinol.

Subcutaneous indole-3-carbinol has been shown to be free of toxic or mutagenic effects at doses at least 80 times those proposed for oral human use [9,15]. However, oral dosage is more relevant to establishing the safety of low dosage DIM as a dietary ingredient. As established above, a substantial proportion of ingested indole-3-carbinol is converted to DIM. Therefore, prior toxicologic investigation at high, oral dosage of indole-3-carbinol is applicable to establishing the safety of supplemental DIM.

A comprehensive toxicologic survey of oral indole-3-carbinol was performed by Shertzer and Sainsbury and supports the safety of low-dose DIM [16]. Indole-3-carbinol, suspended in corn oil, was administered orally to mice. They found no evidence of acute liver damage at doses up to 100 mg per kg. The assessment of behavioral toxicity of an acute dose of indole-3-carbinol showed only minor neurologic signs at a dose of 100 mg per kg. These consisted of reversible changes in activity level (less active), vertical screen test (hangs on but cannot change position), and posture (tends to lean to the right but legs remain in position under body). No neurologic findings were elicited at 50 mg per kg. 100 milligrams per kg represents 29 times the maximum suggested human dose for DIM supplementation. A further margin of safety for DIM was documented when DIM was tested for acute neurologic toxicity as described in section VI., F., "The safety of BioResponse's DIM is supported by in-vitro and in-vivo testing of the product". In this test in rats no neuro-toxicity was demonstrated for DIM even at doses greater than 500 times the maximal suggested human dose. Indole-3-carbinol did show evidence of neuro-toxicity at these high doses in rats, but there was no evidence of neuro-toxicity at the levels claimed to be toxic in mice by Shertzer[16].

A longer-term study of oral indole-3-carbinol by Wortelboer et al. [17], is also relevant to the safety of DIM. They demonstrated the safety of orally administered indole-3-carbinol given at doses of approximately 16 and 40 mgs per kg per day for 28 days. No

changes in body or liver weights were observed compared to controls at these doses (See Table 2 in reference [17]).

Most relevant for the use of DIM as a dietary ingredient are three long-term feeding experiments demonstrating the safety of high dose indole-3-carbinol with chronic use. The first of these, by Grubbs et al. [18], demonstrated the prevention of chemically induced breast cancer in rats by oral indole-3-carbinol given at 50 and 100 mgs per kg for up to two months. Weight gain was normal in both groups and no toxicity was observed at 50 mgs per kg. At 100 milligrams per kg a 33% increase in liver weight was observed with normal histology.

The second and third studies by Kojima et al. [5] and Bradlow et al. [4] involved feeding indole-3-carbinol to rats for a period of two years to prevent spontaneous endometrial and breast cancers, respectively. In these two studies indole-3-carbinol doses varied from 17 to 343 mgs per kg per day. There was no diminished survival even at the highest dose. A possible threshold of chronic toxicity was established at 132 milligrams per kg (1000 parts per million Indole-3-carbinol added to food) which produced a non-significant decrease in body weight in one study [5] and a non-significant trend to increased liver weight in the second study [4]. This threshold is approximately 38 times the proposed maximal dose for DIM of 3.5 milligrams per kg per day.

Most recently, in the study already described by Oganessian et al.[3] indole-3-carbinol was given to mice for a period of 8 months at doses of from 500 to 1500 mg per kg per day with no evidence of toxicity or histologic changes to liver.

E. The safety of the suggested dose of BioResponse's Diindolylmethane is supported by short- and long-term human use of indole-3-carbinol.

Investigation of the safety of oral indole-3-carbinol in humans was first reported in 1990 by Michnovicz and Bradlow [19]. These scientists were the first to establish the effectiveness of a 6-7 mg per kg dose of indole-3-carbinol as a means of promoting estrogen metabolism through the 2-hydroxyestrone pathway. A daily oral

dose was given for 7 days without side effects in seven men. Subsequently, a larger group of men and women was studied for one week at a dose of 5-7 milligrams per kg per day [20]. Again, no side effects were reported.

In 1994 a larger and more definitive study of oral indole-3-carbinol use by women was reported by Bradlow et al. [21]. In this study a group of 20 women received 400 milligrams of indole-3-carbinol per day, averaging 6 mgs per kg. A full battery of safety data including laboratory measurements of liver, renal, and endocrine function were assessed. No changes or side effects were noted except a statistically insignificant rise in total cholesterol from 187 to 198 milligrams per deciliter. Subjective reports from participants were also supportive of safety: "Careful questioning of the patients in the indole-3-carbinol arm revealed no significant differences other than a slight increase in gastrointestinal motility and a decrease in complaints of constipation in a few subjects". The longest daily human use of indole-3-carbinol has been by children and young adults with chronic laryngeal papillomatosis. These individuals have taken approximately 300 mg per day of indole-3-carbinol for periods of up to 5 years with no reported side effects [22].

Compared to 6 mgs per kg per day for indole-3-carbinol, a recommended dose of from 0.5 to 3.5 mgs per kg (1 to 4 capsules per day for women, and 1 to 5 capsules per day for men) will place the BioResponse DIM ingredient well within the range of safety established by investigators using indole-3-carbinol in humans.

F. The safety of BioResponse's Diindolylmethane is supported by in-vitro and in vivo testing of the product.

(The following section describes proprietary technology and trade secrets owned and controlled by BioResponse, and is not for public display or release)

G. Metabolic studies in animals support the safe metabolism of DIM.

BioResponse has provided improved nutrient bioavailability of the DIM ingredient through the means of reduced particle size and microencapsulation with uptake enhancing additives. Steady-state levels for DIM have been established at oral dosage well above the dosage recommended by BioResponse. This was demonstrated in an oral feeding study in rats conducted by Stresser et al. in 1995 [24]. DIM was measured as a gastric conversion product allowing this study of indole-3-carbinol to be used to substantiate DIM metabolism. In this detailed study of indole-3-carbinol disposition and metabolism, DIM was found at a level of 4-6 micromoles per gram of liver in rats fed a dosage of 147 milligrams per kg of indole-3-carbinol for 6 days. Furthermore, evidence for a steady state with active metabolism, allowing stable urinary and fecal excretion of radiolabeled metabolites, was demonstrated at a point 4 days into the study. This evidence is important since the data indicate that animals can maintain a steady metabolic clearance of indole-3-carbinol and DIM at an indole-3-carbinol dosage approximately 40 times greater than the maximum 3.5 milligram per kg dosage recommended for DIM as a dietary ingredient.

Subsequently in 1995, Stresser et al. [25] provided further documentation of the metabolism of DIM. Microsomes from rat liver were directly exposed to radiolabeled DIM, and a monohydroxylated metabolite was isolated. Furthermore, protective enzyme changes were documented at DIM liver concentrations achievable in the 3.5 milligram per kg dosage range for DIM.

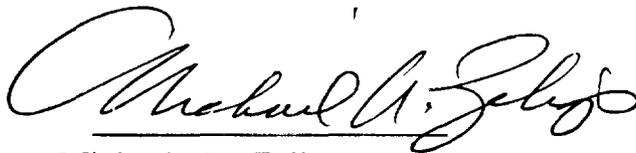
H. Conclusions concerning the safety of BioResponse's DIM.

The BioResponse effort and product design for DIM assures the safety of DIM as a dietary ingredient by introducing a shelf stable product at the lowest possible dosage. BioResponse has adopted a safe means of increasing nutrient bioavailability through its proprietary microencapsulation technology. Rarely have dietary ingredients been brought to the supplement marketplace having

received such intense scientific scrutiny as the dietary indoles. This scientific work supports a role for DIM in improving the safety of estrogen metabolism in men and women. When screened against 90 other natural substances, the DIM precursor, indole-3-carbinol, ranked as one of the eight most promising compounds for promoting beneficial and cell protective effects. [26]. (See table 2, in Sharma et al.[26]) As a dietary indole with a better safety profile than indole-3-carbinol, DIM holds important potential for promoting human health.

Based on prior animal and human use, together with other evidence of safety as a dietary ingredient, DIM has been shown to be safe. Furthermore, the BioResponse DIM dietary ingredient preparation has been shown to be reasonably expected to be safe under the conditions of use outlined above.

VII. RESPECTFULLY SUBMITTED:



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Member
BioResponse, L.L.C.

August 8, 1997

Date

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