DIM and I3C:
The Real Facts on Safety

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Safety First

Two cruciferous indoles -- Diindolylmethane (DIM) and its precursor, Indole-3-carbinol (I3C) -- are increasingly popular as dietary supplements. Researchers have been studying the role of these substances in promoting healthier estrogen metabolism and for potential use in estrogen-related conditions, including cancer. Regarding women's health, use of cruciferous vegetable indoles has importance for breast cancer prevention and for the control of cervical dysplasia. Both DIM and I3C show real promise in these areas, yet studies have also uncovered sharp contrasts between them in one critical area -- *consumer safety*.

Anyone advocating the benefits of I3C over DIM is ignoring the published scientific facts. A careful review of the research reveals serious questions concerning the safety of I3C. For example, the National Institute of Health (NIH) in its Chemoprevention Branch Report on I3C safety issues says: "Subchronic, preclinical toxicology studies in dogs have identified significant toxicities associated with oral administration of I3C… The possible enhancing effects of I3C on chemical carcinogenesis and toxicity should be investigated, especially in light of the wide variety of enzymes induced… The fact that the I3C condensation product ICZ has both antiestrogenic and estrogenic activities also may have safety implications… Additional genetic toxicity testing may be required." (1)

This statement and the published evidence are clear -- I3C, and in particular its reaction product ICZ, are associated with a number of unwanted activities that are not compatible with safe, long-term use. Misinformation raised about DIM and the purported superiority of I3C are simply not supported by the facts.

"…I3C has a mixed reputation — preventing cancer in some experiments, but promoting cancer in others…"

Getting the Facts Straight on Cruciferous Supplements

First, there are many published studies on DIM. There are over 40 studies in the National Library of Medicine Database which involve DIM. In 2001 alone, seven studies were published. In addition, well-controlled, independently-performed human studies have been completed and are awaiting publication. These include use of DIM showing statistically significant benefits for recurrent breast pain and improvement of cervical dysplasia.

Second, ICZ (indolocarbazole) is not a safe derivative of I3C. ICZ activates the dioxin receptor just like dioxin. ICZ does not block it. It is DIM that blocks this receptor (2). This is one of the key benefits of using a stable DIM supplement.

Third, more is not necessarily better. Having a family of reactive products being generated does not provide broader cancer protection if many of these products also have unwanted side effects. Side effects are tolerable in certain cancer treatment drugs for established cancer but not in nutritional substances meant to prevent cancer in healthy individuals.

Fourth, DIM is not estrogenic or a growth promoter of cancer cells when studied in intact humans or animals. When tested in analogous experiments, I3C failed to prevent the progression of cancer (6, 7).

As a physician and a researcher, I have worked with scientific experts around the world to study the chemistry of I3C and to identify its safest and most beneficial form. This led to the identification of DIM as the safest, most active and most beneficial I3C-related substance found in cruciferous vegetables, and to the development of an effective, highly-absorbable DIM supplement.

DIM has been tested in humans. The entire basis of absorbable DIM's introduction as a supplement was based on testing which supported its safe and efficacious use. Many of these studies involve side-by-side testing of DIM and I3C.
In year-long animal tests, independent university-based scientists found evidence of I3C's lack of safety that justified concern -- I3C was found to be highly enzyme-

inducing, but DIM was not. In a second test performed by an independent government laboratory, confirming results again showed unwanted enzyme induction by I3C but not DIM.

Ongoing placebo-controlled human testing is being performed to confirm benefits for DIM observed in cervical dysplasia (40) and breast cancer risk reduction (42). In fact, Leon Bradlow, Ph.D., the original discoverer of the importance of cruciferous indoles for breast health, now supports and advocates absorbable DIM, and not I3C, as the preferred supplement. Dr. Bradlow actually initiated and performed the majority of human and animal testing of I3C.

Recent publications have begun to focus on DIM, recognizing it as more advantageous than I3C. DIM has been shown to be more potent and active than I3C in experimental cervical cancer (3). When inducing cell death (apoptosis) in cervical cancer cells, Dr. Karen Auborn, a renown cancer research scientist, states that "DIM is a more effective inducer of apoptosis than I3C" (3). Other work showed that DIM had a strong anti-proliferative effect in human endometrial cancer cells (4). Indolocarbazole (ICZ), the dioxin-like and enzyme-inducing reaction product arising from I3C, notably failed to control the growth of these endometrial cancer cells (4).

"DIM exerts its control over cancer cell growth without inducing… unwanted enzymes."

Unwanted enzyme-inducing effects from I3C have also been shown in recent animal studies (5). This work clearly demonstrates that oral I3C can increase production of dangerous estrogen metabolites at the same time it increases the beneficial 2-hydroxy metabolites. In effect, this cancels out benefits for estrogen metabolism. Consistent with this, I3C has been shown to be ineffective in controlling the growth of experimental breast cancer (6, 7), while DIM succeeds (8, 9).

Let us now turn to the scientific basis for the NIH's concerns about I3C safety. This involves a discussion of unwanted activity associated with some of the many I3C reaction products (other than DIM) which arise during I3C use.

Putting the Scientific Facts into Perspective

Both I3C and DIM have been studied since the 1970s in experimental models of cancer prevention. The majority of subsequent studies involved I3C, not because it was better, but because it was available and plentiful. I3C, however, is a highly reactive "pro-nutrient" which undergoes complicated reactions once in water or stomach acid (10).

I3C itself has been shown to not leave the stomach or circulate in the blood since it is so reactive (11). Instead, a mixture of I3C byproducts leaves the stomach, some being absorbed, but most remaining in the intestinal tract. These I3C byproducts are *not* the wholesome, well-studied, cancer preventive substances that some wish they were. In truth, I3C has a mixed reputation -- preventing cancer in some experiments but promoting cancer in others (12,13).

Briefly, the reaction products from I3C can be described as follows.

Indolocarbazole (ICZ) -- ICZ resembles dioxin in structure and function (14). Also, like dioxin, ICZ is a powerful inhibitor of estrogen action. Though toxic in many other ways, dioxin has been shown to prevent and treat breast cancer in animals. ICZ is being explored as a possibly less toxic form of dioxin to treat breast cancer. But like dioxin, ICZ does not block the dioxin receptor. It activates it as aggressively as dioxin does (15).

Though a down-regulator of the estrogen receptor system, ICZ is itself estrogenic and is responsible for promoting enzymes which produce 4-hydroxy estrogen, a metabolite associated with uterine tumors (16) Though more quickly metabolized, ICZ shares many of dioxin's toxicities. Like dioxin, ICZ has been shown to be damaging to the thymus gland and immune system (17), and to damage DNA (18).

Importantly, the reaction products arising from I3C have been shown to negatively impact the immune system, resulting in lower natural killer cell activity in animals (19). Like dioxin, I3C and its reaction products have also been associated with reproductive toxicity in animals (20, 25). Unlike I3C, DIM cannot be converted into ICZ in vivo.

Ascorbigen (ASG) -- ASG is the most plentifully produced reaction product from I3C, arising from the combination of I3C with Vitamin C. ASG occurs following consumption of cruciferous vegetables which contain abundant Vitamin C. ASG arises from I3C supplements only when the I3C is taken along with other supplements or foods rich in Vitamin C.

Once produced in the stomach, a portion of ASG is then converted into ICZ in the lower intestine (21). This makes ASG a source for much of the unwanted enzyme promotion attributed to ICZ. This was demonstrated when ASG was fed as a pure compound to animals and measurements of estrogen metabolism showed increased production of unwanted 4-hydroxy estrogen (22).

4-hydroxy estrogen is a carcinogenic estrogen metabolite, increased after activation of the dioxin receptor. While some preliminary work demonstrates anti-cancer activity by ASG in animals, further testing reveals that ASG's natural breakdown results in the release of formaldehyde (23). This makes ASG a highly undesirable I3C product during human use. Other undesirable reactions involving ASG have been described resulting in the formation of DNA damaging mutagens (24).

Linear Trimer (LTR) -- LTR is an anti-estrogen, but clearly another activator of the dioxin receptor. This was recently proven by feeding I3C to pregnant rats and studying the livers of the mothers and their offspring (25). LTR was found in both mother and offspring. Most importantly, the induction of the enzyme source for 4-hydroxy estrogen, CYP 1B1, was also found together with measurable LTR in the rat pup livers. This is evidence that LTR crosses the placenta and activates the dioxin receptor in an analogous way to ICZ in both mother and developing fetus.

Cyclic Trimer (CTR) -- CTR is produced in stomach acid by the condensation of three I3C molecules into a ring structure. CTR is a direct acting estrogenic compound, occupying and activating the estrogen receptor. CTR powerfully stimulates the growth of growth of breast cancer cells, in the presence or absence of estrogen (26).

Indole-3-acetonitrile (IAN) also arises from I3C in the acidic milieu of the stomach. Though shown to be protective in an animal model of stomach cancer, IAN has also been shown to be mutagenic (47) and give rise to DNA-damaging reaction products (27). This occurs when I3C is ingested in the presence of nitrates commonly present in foods (47).

More importantly, IAN is disruptive of cell membranes, activating the arachadonic fatty acid cascade leading to the production of LTB4, a reactive leukotrine (28). Leukotrines are closely related to Lipoxins, which like ICZ, may contribute to further activation of the of the dioxin receptor (29).

There are multiple, additional reaction products from I3C which occur but have been poorly studied. Most of these also resemble ICZ in their activity as activators of the dioxin receptor. Since they are poorly absorbed, this action is especially important for the cells lining the intestines and colon. It is because of this action that I3C is associated with the enhancement of colon cancer in animals (30) and damage to human colon cells in culture (31).

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Conditions have also been studied in animals in which cancer promotion due to I3C was seen in other organ systems (32). With higher pH as seen in the upper intestine and in the stomach with the use of antacid medications like Cimetidine and Zantac, I3C has recently been shown to form a separate, additional set of reaction products (33). In these mild pH environments, I3C results in toxic 3-methylindole leading to DNA-damaging complexes, called adducts (49).

Cell Culture Studies can be Misleading

Much has been made of a cell culture study using DIM which showed that in artificial estrogen-free conditions, DIM contributed to the growth of breast cancer cells (34). This activity, observed only in the complete absence of estrogen, is never seen in living animals where estrogen is always present. In the presence of estrogen, DIM is

consistently anti-proliferative and reduces the growth signal provided by estrogen (35). This includes growth-inhibiting activity in both estrogen receptor positive and negative cancer cell types (36). The estrogen-independent activation pathway described by Riby et al (34) involves the increased phosphorylation of estrogen receptor proteins, and not the direct interaction of DIM with the estrogen receptor. This action may actually be of benefit in cancer control.

Understood in context, this receptor activation pathway contributes to the control of abnormal cell growth in living animals through enhanced "programmed-cell-death" (apoptosis) and a reduced tendency for the metastatic spread of cancer (37).

Unlike ICZ, LTR, CTR or ASG, *DIM exerts its control over cancer cell growth without activating the dioxin receptor or inducing unwanted enzymes* (38). Direct control over cancer cell growth by DIM has now been shown in breast, uterine, cervical, ovarian, and colon cancer cells. I3C has been noted to control cancer cell growth in these cell types as well. However, in cell culture media at 37 degrees Centigrade, most of the I3C is converted to DIM in 24 hours (39). Since no ICZ and the other condensation products formed in gastric acid occur in cell culture media, DIM may very well be responsible for much of the activity attributed to I3C in prior cell culture studies.

Animal and Human Studies

Apart from the cell culture data, in vivo use in animal models and clinical use in humans carries the most importance in deciding safety and efficacy. DIM is the only cruciferous indole which treats established breast cancer in animals. This has been shown in separate studies by different investigators (8, 9). Giving I3C as the supplement failed to control breast cancer growth in an equivalent study (6).

Regarding human studies, *DIM has demonstrated every benefit known to accrue to I3C use*. This includes shifting estrogen metabolism to produce more protective 2-hydroxy metabolites. DIM also has shown the same benefit as I3C in helping to resolve cervical dysplasia. These studies will soon be presented and published by the American Association of Cancer Research (40).

"… higher doses of DIM are well-tolerated, unlike I3C, where higher doses produce side effects such as dizziness and unsteady gait."

Plus, higher doses of DIM are well-tolerated, unlike I3C, where higher doses produce side effects such as dizziness and unsteady gait (41). Higher doses of absorbable DIM are being used in monitored studies of cervical dysplasia, in combined use with Tamoxifen, and in adults and children with viral papillomas. This monitored use of high dose absorbable DIM involves before and after testing of liver function in human subjects and has revealed no abnormalities.

This demonstrated lack of side effects from high doses of absorbable DIM further underscores its safety when used at lower doses as a dietary supplement. Currently, formal placebo-controlled studies using absorbable DIM are in progress for cervical dysplasia and recurrent breast pain in women. Open label studies without placebo are being conducted for cervical dysplasia, cutaneous and plantar warts, and laryngeal papillomas.

These human studies, often at ten times the typical supplement dose of DIM which benefits estrogen metabolism, have reported no side effects. More detailed human studies involving DIM are planned to further examine its safe use in humans. This includes a study approved at UC Berkeley in women with breast cancer where absorbable DIM will be given to improve estrogen metabolism and lower cancer risk status (42).

In contrast to this, a small trial of I3C in humans demonstrated nausea and vomiting in 25% of subjects when the typical dietary supplement dose of 400 mg/day was tripled to 1200 mg (43).

What about the Vitamin-E TPGS in the patented DIM formulation?

In absorbable DIM, pure (but poorly soluble) DIM is complexed with Vitamin-E TPGS to provide for and enhance DIM's absorption. Vitamin-E TPGS (Tocophersolan) is a well-known ingredient in foods and pharmaceuticals. It is so safe as to appear on the Generally Regarded As Safe (GRAS) list maintained for ingredients by the FDA.

Rarely are dietary supplement ingredients safe enough to appear on this list. Proof of its safety has been established in extensive testing, including testing by the NIH (44). Vitamin-E TPGS is also used to improve the absorption of Vitamin-D (45) and Vitamin-E (46) in infants.

DIM is Effective and Safer than I3C

Use of absorbable DIM has been shown effective in amounts close to that obtainable from our diet (0.3 mg/kg/day of DIM) (40). I3C requires about 15 times more than this (4.5 mg/kg/day (48)), and is associated with side effects (41,43). In preventive nutrition, even small risks and potential toxicities must be taken seriously, lest the negatives outweigh the positives and cause harm.

The I3C safety issues described here should not be lightly dismissed -- every one of these safety concerns must be addressed by anyone advocating I3C. Animal and human tests raise the concern of I3C-related toxicity. Excessive enzyme induction, as seen with I3C, is associated with disease promotion.

In contrast, DIM has a consistently positive safety history which makes a strong argument for its safe, effective and long-term use in humans.

"…DIM provides distinct advantages over I3C. DIM is a conservative, well-studied, safe and clearly effective natural substance."

For those interested in a scientifically-based, cruciferous supplement for estrogen-related concerns, DIM provides distinct advantages over I3C. DIM is a conservative, well-studied, safe and clearly effective natural substance.

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For more information, please visit the "DIM FAQ" web site at: http://www.dimfaq.com

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