Paraben Toxicology

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Parabens now being formally declared as the American Contact Dermatitis Society (non)allergen of the year, the allergologic concerns regarding parabens raised during the past century are no longer a significant issue. The more recent toxicological concerns regarding parabens are more imposing, stemming from the gravity of the noncutaneous adverse health effects for which they have been scrutinized for the past 20 years. These include endocrine activity, carcinogenesis, infertility, spermatogenesis, adipogenesis, perinatal exposure impact, and nonallergologic cutaneous, psychologic, and ecologic effects. To assert that parabens are safe for use as currently used in the cosmetics, food, and pharmaceutical industries, all toxicological end points must be addressed. We seek to achieve perspective through this exercise: perspective for the professional assessing systemic risk of parabens by all routes of exposure. The data reviewed in this article strive to provide a balanced perspective for the consumer hopefully to allay concerns regarding the safety of parabens and facilitate an informed decision-making process. Based on currently available scientific information, claims that parabens are involved in the genesis or propagation of these controversial and important health problems are premature. Haste to remove parabens from consumer products could result in their substitution with alternative, less proven, and potentially unsafe alternatives, especially given the compelling data supporting the lack of significant dermal toxicity of this important group of preservatives.

Parabens are safe and effective as biocides from preservation and cutaneous allergologic standpoints. With the remarkably low and predictably stable patch test results of allergic reactions reported in screening series, and with the worldwide application of use concentration guidelines and restrictions, there are no scientific data to suspect that this interpretation will change in the future. This is true despite extensive exposure, largely cutaneous but also through foods and medications.[1]

Although both parenteral and cutaneous routes result in systemic exposures, parabens are rapidly metabolized to *p*-hydroxybenzoic acid and rapidly excreted in the urine.[2] This would suggest that little or no systemic hazardous biological effect is possible because of their remarkable short half-lives when absorbed through skin or taken systemically. In human studies, urinary excretion of parabens peaks within 7 hours of taking paraben-containing medications; in the rat model, urinary excretion of paraben metabolites begins within 30 minutes of oral dosage and peaks at 90 minutes.[3,4] However, chronic administration of a substance through the regular application of paraben-containing topical products and/or regular ingestion of medications or foods with paraben preservation could theoretically result in a steady-state phenomenon where lasting, albeit low, plasma and tissue levels are maintained. If biological activity is confirmed and a substance is demonstrated to be noninert, a biological effect may result (the nature of the effect dependent on what biological capabilities that substance possesses). Late in the 20th century, parabens were demonstrated to have estrogenic potential, which raised initial concern regarding their safety but not to the level where guidelines for use were modified. In 2004, parabens were identified in human breast tumors by Darbre and co-investigators.[5] This report and a series to follow have fueled the controversy regarding paraben safety; the medical and scientific communities continue to examine exactly what significance, if any, these findings represent to human health. The goals of this review article are to examine the concerns that have arisen, assess the strength of investigative data regarding each concern, and gain perspective regarding the probability of any significant role (initiation and propagation) that parabens may have on each of the purported adverse health effects and the potential risks to human health under the current conditions of use.

There are many purported adverse health effects, which may alarm consumers regarding the use of paraben-containing food, medicinal, and cosmetic products. These include endocrine disruption, carcinogenicity, teratogenicity and reproductive toxicology, and adipogenesis as based on animal data. In addition, there are concerns regarding potential perinatal and childhood exposure risks, psychosocial issues, and environmental effects. Although there is overlap in several of these effects, this review will cover each adverse health effect separately to provide a clear and reasonable justification regarding a cause and effect related to parabens. However, the most significant

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areas of concern with respect to parabens involve potential endocrine mimicry and the putative association with developmental anomalies and the development of breast cancer.

PARABEN PENETRATION THROUGH HUMAN SKIN

Cosmetic product application has been identified as the major source of aggregate human exposure to parabens according to a study by the Danish Environmental Protection Agency (EPA); however, the question remains as to whether it is the major source of systemic exposure that would account for the toxicological end points addressed in this study.[6] The absorption of parabens is highly influenced by skin integrity and barrier function, with minimal penetrance through normal intact human skin.[7] Despite the differential ability that parabens have to penetrate intact human skin (dependent on structure and side chain characteristics) and the reported dermal uptake of parabens that may occur, metabolism (largely hepatic) is very efficient with little intact parabens found in blood and urine.[8]

The cutaneous absorption of parabens is highly vehicle dependent. An in vitro epidermal membrane diffusion study has shown, predictably, that occlusion with the volatile solvents acetone and ethanol increases penetration; although decreased penetration was seen for occluded ointment vehicles, the authors postulated that partitioning and diffusion within the epidermal membrane due to occlusion explained the phenomenon.[9] Filaggrin is an important epidermal protein for barrier function integrity, with 10% of Europeans and Asians having loss of function mutation resulting in significant transepidermal penetration of a number of chemicals. In Danish men, those with abnormal filaggrin averaged 80% and 91% higher absorption for methyl- and *n*-propylparaben, respectively, than controls as evidenced by measurements of urinary excretion.[10]

Pažoureková and colleagues[11] determined that, after dermal administration, a certain amount of methylparaben goes unmetabolized and may remain systemically available, while damaged skin may facilitate absorption. As much as 923 μg/kg of body weight per day of unhydrolyzed methylparaben was shown to be systemically absorbed after the application of a leave-on emulsion containing methylparaben to damaged skin. Thus, a healthy skin is essential to barrier function.

When considering the sex difference in cosmetics utilization, not surprisingly, urinary concentrations in women are twice those found in men.[12] The nature of the product must be factored in, for example, moisturizers applied to larger body surfaces and leave-on products having prolonged contact time, thereby increasing the possibility of absorption. Because parabens with shorter side chain groups have greater skin and dermal penetration potential due to lipophilicity, methylparaben has been demonstrated to be absorbed at a higher rate than other parabens.[13]

CHRONIC EFFECTS ON HUMAN SKIN

Human and animal studies have failed to show that parabens have any acute toxicity by any route of administration; most published studies have concentrated on long-term chronic effects.[14]

Although a great deal is known about penetration of parabens through human skin and the metabolism of absorbed parabens, much less is known about the effects parabens have on the skin itself. Parabens have been deemed relatively nonirritating at levels used in current formulations, as verified in extensive experience with the mix at current applied patch test concentrations by the North American Contact Dermatitis Group.[1] This claim is supported by data from an epidemiologic study of 4226 healthy subjects who use-tested 151 different paraben-containing formulations (along with other ingredients); a tendency toward decreased irritation was actually identified.[15] In this retrospective analysis, 1363 cumulative irritation test studies in more than 45,000 subjects did not demonstrate parabens to be irritating in typical in-use conditions and irritation scores did not correlate with preservative concentrations.

The role of the cutaneous microbiome in health and disease is receiving increasing attention.[16] The skin is an immunological organ as well as a physical barrier.[17] As an interface immunologic defense system, it is important for normal wounding healing and infection prevention. Keratinocytes monitor these defenses through pattern recognition receptors (toll-like receptors, mannose receptors, and the nucleotide-binding oligomerization domainlike receptors), which recognize pathogen-associated molecular patterns including flagellin, nucleic acids, lipopolysaccharides, and peptidoglycan.[18] Activated keratinocytes initiate the innate immune response, resulting in the secretion of antimicrobial peptides, cytokines, and chemokines, which directly kill bacteria, fungi, and enveloped viruses. It is logical that topically applied microbicides may adversely impact this natural immune bacterial balance, but studies have shown little or no direct impact on the ecology of the microflora; however, continued surveillance of preservative effects on skin microbiome has been called for because of the flexible and selectively adaptive nature the microbiome exhibits.[19,20] Cosmetic use in "western skin" has been implicated as decreasing microbiota diversity in comparison with "unexposed skin," suggesting a less healthy and less effective ecosystem.[21] Conversely, another study demonstrated increased diversity of bacterial species after cosmetics use regardless of hydration status.[22] The complexity of both microbiome and cosmetics and toiletries formulations makes characterization and interpretation of the effects that specific biocides such as parabens may cause on this ecosystem problematic.

Daily application of methylparaben to human skin for 1 month decreased keratinocyte proliferation, changed cellular morphology, and decreased the expressions of hyaluronan synthases 1 and 2, messenger RNAs (mRNAs), and type IV collagen; in contrast, involucrin and HSP27 levels were increased.[23] The authors speculate that, because methylparaben was detected unmetabolized and slightly persistent in the stratum corneum, accumulation caused by routine cosmetic product application could impact keratinocyte differentiation and aging. Other cellular changes may occur with paraben exposure, with methylparaben-treated cells experiencing cell cycle G2/M arrest, which could lead to cellular senescence if DNA damage goes unrepaired.[24] Handa and colleagues[25] determined that 15- to 30-mJ/cm^2 UV-B exposure of 0.003%

methylparaben-treated HaCaT keratinocyte cultures enhanced UV-B–induced cell death (as assessed by immunocytochemistry and flow cytometry). These data could raise concern regarding the presence of parabens in sunscreens and their potential chronic solar-induced cutaneous effects. However, one could easily argue that increased keratinocyte cell death would be photoprotective for cutaneous carcinogenesis, if the cells primarily affected are those that have undergone tumor promotion.

ESTROGENIC/ANTIESTROGENIC EFFECTS

Parabens have been demonstrated to be rapidly metabolized and excreted in urine, with a biological half-life of less than 24 hours. Their conversion by esterases in the skin and liver is efficient, and the primary metabolite, *p*-hydroxybenzoic acid, has significantly weaker xenoestrogenicity. That parabens are weakly estrogenic seems to be well supported by existing laboratory and animal study data; the claim of in vivo endocrine disruption (clinical interference with normal endocrine function) in light of the remarkable efficiency of cutaneous and hepatic esterases is less substantiated.

There is a growing body of evidence implicating a wide variety of substances as negatively impacting normal endocrine function, with data available supporting both estrogenic and antiestrogenic effects.[26] Many lists of confirmed and potential chemicals with endocrine activity have been assembled, with inconsistent scientific data in support of these claims of potential health hazard. There are numerous epidemiological studies and case reports with a range of methodologies used and cohorts evaluated. Randomized clinical trials, of which there are few, represent the apex of the evidence pyramid.[27] This is followed in decreasing strength of argument order by prospective cohorts, retrospective cohorts, case-controlled study, cross-sectional study, and case reports. The number of studies that have examined the estrogenic potential of parabens with randomized clinical trials is few, often with variable results.[28,29] The number using prospective cohort and cross-sectional techniques is greater.[30] This demands caution when assessing the strength of data and interpretation from these studies of different designs.

The European Union (EU) catalogs an exhaustive list of chemicals with estrogenic potential, maintained by the Ministry of Environment and Food in Denmark.[31] In all, there are 194 such substances on this compendium, with methylparaben, ethylparaben, propylparaben, and butylparaben meriting inclusion, along with the by-product of their in vivo metabolism, *p*-hydroxybenzoic acid. The categories of chemicals suspected of possessing endocrine activity are stratified according to scientific level of risk confirmation.[32] In all, there are 3 categories, with the complete listing tabulated in EU Annex X.[33] The 4 parabens previously mentioned and *p*-hydroxybenzoic acid are found in category 1, where listed chemicals have been shown to exhibit evidence of potential endocrine disruption (and thus specified as an endocrine disruptor compound [EDC]). Ketoconazole, resorcinol, and active sunscreen ingredients 2-ethylhexyl 4-methoxycinnamate (octinoxate) and methylbenzylidene camphor (Eusolex 6300) are also listed in category 1. Category 2 (evidence of biologic activity but no evidence of endocrine disruption) includes 2-hydroxy 4-methoxybenzophenone (oxybenzone). Category 3 represents the remainder of the Annex X, with subdivision "a" for substances for which there are no indications of endocrine-disrupting properties and "b" for substances for which no or insufficient data have been gathered (n = 109). Metals and carbamates are notable in this list, including aluminum, cadmium, copper, lead, and mercury. All 3 categories are dominated by insecticides, pesticides, herbicides, and other chemically active microbicides. Additional substances implicated as potential endocrine disruptors in cosmetics include aluminum salts, *p-tert*-butylphenol, and triclosan. Interestingly, isopropylparaben (IPP) and isobutylparaben (IBP) are listed in many publications as "known endocrine disruptors," yet they are not included in the EU endocrine disruptor category 1 grouping.

The assertions that parabens exhibit endocrine activity are based on in vitro and animal in vivo evidence.[6,8,34] The strength of the data in support of this assertion has been questioned, however, based on the nature of the studies published. As "weak estrogens," parabens have been demonstrated to possess a small fraction of the activity of native estradiol; it is believed that all of the commercially used parabens possess varying degrees of estrogenic potency, hence their inclusion as EDC.[2] Routledge and colleagues[35] first reported estrogenic activity of paraben moieties in 1998. They also confirmed the low potency of parabens with respect to xenoestrogenicity; they estimated the 4 most commonly used parabens in cosmetic products to be 10,000-fold less potent (butylparaben) or lower (methylparaben, ethylparaben, propylparaben) in comparison with 17β-estradiol. The authors concluded that "the safety in use of these chemicals should be reassessed, with particular attention being paid to estimation of the actual levels of systemic exposure in humans. The acquisition of such data is a prerequisite to the derivation of reliable estimates of the potential human risk of exposure to parabens." This conclusion was supported by the data from a 2001 report, where a recombinant yeast assay was used.[36] Cells transfected with the human estrogen receptor (ERα) gene together with expression plasmids (containing estrogen-responsive elements) were incubated with benzylparaben, butylparaben, propylparaben, ethylparaben, and methylparaben to determine their estrogenic activity in comparison with estrogen (17β-estradiol). There was a progressive increase in the estrogenic activity with increasing molecular weight, with methylparaben, ethylparaben, propylparaben, butylparaben, and benzylparaben having a relative potency to estrogen of 1/3,000,000, 1/200,000, 1/30,000, 1/8000, and 1/4000, respectively.

When an estrogenic substance binds to and activates the ER (presumably including xenoestrogenic derivatives), activation may result in conformational changes, protein interactions, and gene transcription.[37–39] The potential to result in meaningful transcriptional (and conformational) change is believed proportional to the avidity and affinity of a substance to bind specific receptors; binding does not necessarily result in activation, and binding avidity does not necessarily correlate with degree of transcriptional activity identified. In their comprehensive study of 188 natural and xenochemicals, Blair and colleagues[40] found diethylstilbestrol to be the most potent

binding substance (399.56 relative affinity) with paraben affinities between 4.5^{-5} and 1.0^{-6} as potent (relative activity, 0.018–0.0004). As identified in many other studies, they determined that estrogenic strength increased in proportion to the length of the alkyl side chain; 2-ethylhexyl paraben was bound with the greatest avidity, whereas methylparaben showed the weakest binding capacity.

Darbre and Harvey[41] reviewed the data on estrogenic activity of 8 parabens and the metabolite *p*-hydroxybenzoic acid in more than 25 studies between 1998 and 2007. Many reported binding activity to some degree in yeast + receptor binding, human Michigan Cancer Foundation-7 (MCF-7) (referring to the institute in Detroit that established the adenocarcinoma cell line from a 69-year-old white woman) cell lines, human Henrietta Lacks cell line overexpressing ER, and human breast cancer cell line ZR-75-1 in vitro; mixed but frequently positive results were reported for in vivo studies using mouse and rat uterotrophic systems. In response to the argument that parabens are weak estrogens at best, these authors stated that the distinction must be made between ligand affinity and ligand efficacy, as justification why simple affinity models as described previously may not reflect the actual biological impact of xenoestrogenic substances. This argument, notwithstanding the actual biological outcome demonstrated by these specific in vitro and laboratory animal in vivo studies, lacks confirmation in human study. The same authors assert that paraben estrogen mimicry is variable; parabens seem to activate a certain few genes, whereas the activation from estrogen is more widespread (hundreds of genes upregulated). It is known that estrogen is ubiquitous in our species and has necessary physiologic roles in everything from conception to sexual characteristic maturation; binding of a chemical substance to a receptor with (or without) transcriptional and gene upregulation activity does not necessarily result in a significant, much less deleterious, in vivo biological effect.

Fang and colleagues,[42] using estradiol as a template, examined structure-activity relationships for a total of 230 chemicals and identified 5 distinguishing criteria essential for xenoestrogen activity (Table 1). The very weak binding ability of chemicals may correlate to the lack of or variable fulfillment of up to 3 of these 5 characteristics.

The optimal investigational system for the assessment of estrogenic potential has not yet been identified; the interested reader is referred to the following 2 comprehensive sources.[43,44] The Food and Drug Administration (FDA) maintains the Estrogen Activity Database, which contains 18,114 estrogenic activity data points collected for 8212 chemicals tested in 1284 binding, reporter gene, cell proliferation, and in vivo assays in 11 different species. Using this database, a group of FDA investigators studied the techniques used to assess estrogenic potential and found variable but generally high concordance between investigation techniques; they recommended that a group of assays, rather than one technique, be used.

The mechanism of xenoestrogenicity of parabens is not well understood. Paraben esters are rapidly metabolized in vivo to the relatively inactive metabolite *p*-hydroxybenzoic acid, and to what extent micromolar concentrations of parabens disturb normal estrogen activity or function as mimicker in vivo is unknown. One hypothesis relates the potential biologic activity to the inhibition of enzymatic activity.[45] These authors determined that all parabens inhibit 17β-hydroxysteroid dehydrogenase type 2 at micromolar concentrations, with 50% inhibitory concentration between 1 and 10 μM; this level of inhibition of enzyme activity would increase conversion of estradiol to the weaker estrone. Similar ranges of micromolar concentrations also inhibit 17β-hydroxysteroid dehydrogenase type 1 in a size-dependent fashion at roughly the same level, with heptylparaben and hexylparaben as the most active (increasing estrogenic activity). The balance of inhibition of these counteracting enzymes that would dictate the biological effect is dependent on paraben side chain size (Fig. 1).

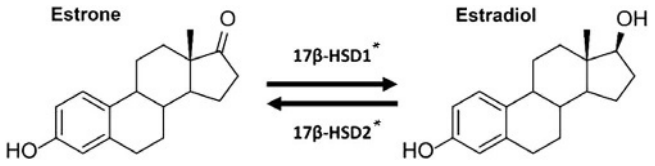
TABLE 1. Structure-Activity Relationships of Parabens

Criteria No.	Characteristic Identified	Paraben Characteristics
1	H-bonding ability of the phenolic ring mimicking the 3-OH	Uncertain activity
2	H-bond donor mimicking the 17β-OH and O-O distance between 3- and 17β-OH	Uncertain activity
3	Precise steric hydrophobic centers mimicking steric 7β and 11β substituents	Uncertain activity
4	Hydrophobicity	Varies; decreases with increasing alkyl side chain
5	Ring structure	All parabens have a ring structure.

Other studies suggest paraben effects on mRNA levels and protein expression of (ER)-a (ESR1) and (ER)-b (ESR2) and the progesterone receptor (PGR). Methylparaben, propylparaben, and butylparaben incubated with human MCF-7 breast cancer and MCF-10A nontransformed epithelial cells showed differential and variable stimulation of mRNA levels and protein expression via PGR stimulation; all parabens stimulated ESR1 expression in both cell lines (except butylparaben in MCF-10A cells).[46] The investigators conclude that, via these effects on hormone receptor expression and stimulation, the estrogenic effect of parabens and putative initiation and progression of breast cancer may be explained.

Van Meeuwen and colleagues[47] found that parabens inhibit aromatase, an enzyme involved in a rate-limiting step in steroidogenesis at one order of magnitude less than that which induces MC7 human breast cancer cell line proliferation, potentially indicating a more potent antiestrogenic effect. However, their calculation of extra estrogen burden due to exposure to parabens in combination with phthalates and polycyclic musk derivatives indicated an insignificant estrogenic load for all 3 combined in comparison with that of the endogenous or therapeutic estrogenic burden.

It should be noted that binding and uterotrophic data have demonstrated that fertilized egg implantation has not been shown to be



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*HSD = Hydroxysteroid Dehydrogenase

Figure 1. Competitive enzyme activity and estrogen derivative potency. Reproduced and modified from Engeli et al,[45] with permission. HSD indicates hydroxysteroid dehydrogenase.

prevented, often considered the most sensitive measure of chronic estrogen toxicity.[48,49]

It is important to consider the host in addition to the eliciting chemical(s) when considering biological risks. Critical periods of developmental sensitivity exist to endocrine active agents including natural and synthetic hormones.[50] The fetus and young children are considered to be most vulnerable to EDC exposures, which is the rationale behind the EU decision to ban parabens in products intended to be applied to intertriginous areas in children younger than 3 years.[51] The older population may also be more sensitive to the potential for endocrine disruption, because metabolism decreases with age.[52]

Conversely, evidence exists that there could be beneficial effects from xenoestrogenic activity that parabens may or may not exhibit in vivo. As stated previously, parabens have been shown to inhibit skin estrogen sulfotransferase activity, as measured in human skin cytosolic fractions and normal human epidermal keratinocytes.[53] Structure-activity relationship study reveals greater activity against estradiol with increasing ester chain length (similar to ER affinity size effect) and with no effect for dehydroepiandrosterone. The authors identify the potential impact of such activity on the antiaging effects of paraben-containing cosmeceuticals.

Summarizing the previously mentioned information, it would appear that, although theoretical concerns regarding paraben activity and xenoestrogenicity are supported by a body of in vitro and animal in vivo evidence, the actual impact, if any, to human health is far from clear, especially given the margin of exposure safety data reviewed previously. Thus, it is not surprising that there are no studies in humans confirming harmful effects of paraben exposure from the estrogen mimicry standpoint.

CARCINOGENICITY

Breast Cancer

Parabens are inactive in classical assays for mutagenicity and carcinogenicity.[54] The role of estrogens in the initiation of breast cancer, as well as their impact on growth, progression, and metastasis, and the salutory effect of estrogen blockade on progression are well documented.[55,56] Additional documented factors increasing breast cancer risk include early menarche, late menopause, delayed onset of childbearing, family history of breast cancer, genetic susceptibility, history of ionizing radiation exposure, excessive alcohol consumption, and prolonged use of estrogen and progesterone in postmenopausal women.[57] When it became apparent that parabens exhibit theoretical estrogen mimicry capabilities in 1998, and with estrogen implicated in breast cancer causation, interest in the xenoestrogenic impact of parabens (and other chemicals) in cancer causation gained momentum.

Estrogenic activity of parabens in MCF-7 human breast cancer cells was first identified by Byford and colleagues,[58] who noted that parabens in concentrations that could be found in vivo were adequate to do so. Wróbel and Gregoraszczuk[46] confirmed the effects of parabens at the level of trophic receptor effect and protein transcription; consistent increase in ER1 mRNA levels and protein expression in both benign (MCF-10a) and malignant (MCF-7) cell lines was identified at 20-nmol/L concentration. Variable effects were seen on PGR and ER2 mRNA levels and protein expression between various paraben esters and cell lines.

Darbre and colleagues[5] demonstrated the presence of intact paraben esters in human breast tumors. Subsequent studies have determined that paraben derivatives may be present in human breast cancer tissue at concentrations comparable with those shown to stimulate proliferation of estrogen-responsive breast cancer cells in vitro.[41,59] A follow-up study by Charles and Darbre[60] examined the stimulatory thresholds that parabens exhibit for the human breast cancer cell line MCF-7; these were then compared with concentrations identified in 160 human breast tissue samples. Twenty-seven percent of the tissue samples were shown to have at least 1 paraben at a concentration greater than that of the lowest-observed-effect concentrations in the cell line. The proliferative stimulus was found to be heightened by using combinations of 5 paraben esters at concentrations down to the 50th percentile in the breast tissue samples examined and suggested that a combination trophic effect may exist. Measurement of the presence of biological effects of all 5 parabens rather than isolated esters was advised.

Although cosmetic products in general and underarm hygiene products in particular have been suggested to be the source of the parabens found in benign and malignant breast tissues, Barr and colleagues[61] evaluated the presence of 5 parabens in 4 serial locations across the breast in 60 women undergoing mastectomy for breast cancer. One or more paraben esters were quantifiable in 158 of the 160 tissue samples (99%), and in 96 of the 160 tissue samples (60%), all 5 esters were present; the highest levels were found in the axillary regions (traditionally higher anatomical risk for breast cancer than in the other regions). This was found even in women who had never used underarm deodorant, and the authors speculated that paraben transport to breast tissue may occur from other nonpercutaneous sources.

In 2011, Hanahan and Weinberg[62] identified 6 biological capabilities as hallmarks of cancer promotion. They include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and

activating invasion and metastasis. Underlying these hallmarks are genome instability, which generates the genetic diversity that expedites their acquisition, and inflammation, which fosters multiple hallmark functions. Conceptual progress in the past decade has added 2 emerging hallmarks of potential generality to this list: reprogramming of energy metabolism and evading immune destruction.[62] Darbre and Harvey[63] contend that parabens can facilitate cancer development in human breast epithelial cells by meeting the criteria for 4 of the following 6 basic hallmarks: (1) being present in 99% of human breast tissue samples, possessing estrogenic activity that could possibly stimulate sustained proliferation of human breast cancer cells at concentrations measurable in the breast; (2) inhibiting suppression of breast cancer cell growth by hydroxytamoxifen and, through binding to the estrogen-related receptor γ, preventing deactivation by growth inhibitors; (3) providing dose-dependent evasion of apoptosis in high-risk donor breast epithelial cells; and (4) long-term exposure (>20 weeks) to parabens leading to increased migratory and invasive activity in human breast cancer cells, properties that they link to the metastatic process.[63] As an emerging hallmark, the authors state that methylparaben has been shown in human breast epithelial cells to increase mammalian target of rapamycin, a key regulator of energy metabolism. "As an enabling characteristic parabens can cause DNA damage at high concentrations in the short term but more work is needed to investigate long-term, low-dose mixtures."

It is important in reviewing these data to note that the concentration of estradiol in normal human breast tissue has been reported to be 44.3 pg/g, suggesting that there is a safety margin of 10 to 1000 times for parabens to approximate normal estradiol activity.[61,64,65] Although parabens mimic the activity demonstrated by the naturally occurring hormone estrogen, the magnitude of activity (ie, potency) is substantially lower for parabens and the potential to result in an adverse effect mediated via an estrogen mode of action has not been established in humans.[64] Golden and colleagues[65] go on to assert that "based on these comparisons using worst-case assumptions pertaining to total daily exposures to parabens and dose/potency comparisons with both human and animal no observed-effect levels and lowest-observed-effect levels for estrogen or DES, it is biologically implausible that parabens could increase the risk of any estrogen-mediated endpoint, including effects on the male reproductive tract or breast cancer."

Parabens are not the only chemical family implicated in the xenoestrogen-oncogenicity connection. The deleterious health effects that parabens have been claimed to possess have also been documented for a large number of other chemical substances; relative risk assessment needs further study if clarity in pathogenesis is to be gained. Darbre, the same principle author whose 2004 publication first raised serious concern regarding paraben exposure and breast cancer risk,[5] has published numerous articles regarding many other "xenoestrogens" found to either be present in or have the potential to accumulate in normal human and cancerous human breast tissue and to show stimulatory effects at identified concentrations. These include the chemical ultraviolet filters benzophenone 1 to 3 (oxybenzone), octylmethoxycinnamate, 4-methylbenzilidenecamphor, and homosalate via different mechanisms and with variable strength of effect[66,67]; methylsiloxanes in personal care products (hexamethylcyclotrisiloxane [D3], octamethylcyclotetrasiloxane [D4], or decamethylcyclopentasiloxane)[68]; aluminum in diet, antacids, and deodorants[69–76]; environment chemicals with estrogenic activity including organochlorine pesticides and polychlorinated biphenyls (including 3,4,3',4'-tetrachlorobiphenyl)[77,78]; benzyl salicylate (fragrance and UV light absorber), benzyl benzoate (fragrance and preservative), and butylphenyl methylpropional (lilial or *p-tert*-butyl-α-methyl hydrocinnamic aldehyde, fragrance)[79]; phytoestrogens including resveratrol[80,81]; metalloestrogens including aluminum, antimony, arsenite, barium, cadmium, chromium cobalt, copper, lead, mercury, nickel, selenite, tin, and vanadate[82]; and triclosan.[83] Conversely, the same investigative center reported an inhibitory effect for breast cancer cell growth for retinoids and, specifically, retinoic acid[84,85] and tocotrienols from vitamin E.[86] These numerous potential xenoestrogenic exposural risks have been discussed in other reviews, in addition to bisphenol A, alkylphenols, and glycol ethers, and indeed all of Annex X and beyond.[87–89] Much like the paraben family, if there is a biological effect from any or all of these chemical substances on oncogenesis due to frequent human exposure, the roles they play are far from clear.

Darbre and Charles[90] addressed this particular issue in a 2010 article, stating that the combination of multiple potential xenoestrogenic and cancer-promoting chemical exposures may be necessary; the process may require multiple such exposures, perhaps in lower doses than those that would cross the threshold of carcinogenesis independently but could potentially pose risks with adequate, costimulatory effects when present together in human tissues. This was referred to in previous publication as the potential for these multiple biologically active exposures to add to the "burden of aberrant estrogen signaling" within the human breast; it simultaneously also underscores the lack of clarity regarding any cause-and-effect relationship between parabens and human malignancy.[82] There are several publications in support of their hypothesis.[91,92]

There is no question that we are exposed to a very large number of chemicals directly or indirectly every day; it has been estimated that more than 80,000 chemicals are manufactured in or imported into the United States each year. Determining what single substance or combination of chemical exposures physiologically produces what effect resulting in induction of what disease process, if any, is a difficult study to design and even more difficult to control.[50]

There are concerns and questions that remain regarding the studies that have been performed to date and their application to humans. Most important is the paucity of clinical confirmation to substantiate these hypothetical claims. For example, Mirick and colleagues[93] found no increased risk of breast cancer incidence in 813 patients compared with 793 controls when examining antiperspirant, deodorant, shaving technique, or application of (presumably paraben-containing) products within 1 hour of shaving. After Darbre's 2004 report, regulatory authorities are heavily involved in the paraben controversy. The European Commission's Health & Consumer Protection Directorate General formed a scientific committee to review the

evidence against parabens. The committee found a number of problems with the Darbre study, including the fact that most underarm cosmetics do not contain parabens; they concluded that "there is no evidence of demonstrable risk for the development of breast cancer caused by the use of paraben-containing underarm cosmetics."[94] This opinion was reiterated in 2006, 2008, and 2011 publications, because either no significant interim evidence had been presented to reevaluate this opinion or that submitted information had "too many shortcomings to be considered as scientifically valid…."[95,96] In the 2011 report, they affirmed that methylparaben and ethylparaben are not subject of concern and that "the safety assessment of propyl- and butylparaben cannot be finalised yet." The same organization confirmed the weak estrogenic potential of parabens but reiterated that, with the xenoestrogenic potency of parabens several powers of magnitude less than that of estradiol, the risks from current cumulative exposure are minimal. This and other regulatory and supervisory entities such as the FDA and the Cosmetic Ingredient Review anticipate further work in the field of skin penetration/metabolism and pharmacokinetics to confirm or refute existing data, and the Scientific Committee on Consumer Safety specifically requested supplementation of envisaged studies in the rat with toxicokinetic studies in human volunteers after dermal application of representative cosmetic products containing propylparaben and butylparaben. When pressed on the relevance of the presence of parabens in human breast cancer tissue, Darbre is on record as responding that "nowhere in the manuscript was any claim made that the presence of parabens had caused the breast cancer, indeed the measurement of a compound in a tissue cannot provide evidence of causality."[65]

Another study design concern is the subtle differences in cell cultures from different laboratories.[97] Michigan Cancer Foundation-7 cells are used worldwide; because the passage number for each culture is likely to differ between laboratories, the degree of variation introduced into research results generated using this cell line is brought into question.[98,99] Hence, reported results must be reproducible and validated.

With epidemiologic evidence lacking, there is also the question of persistence of parabens in human tissues over time and their long-term effect in vivo rather than short-term biological activity demonstrated in vitro. The absolute number of tissues and tumors that have been examined remains small, and some studies have not been adequately controlled. Furthermore, geographic differences in paraben exposures internationally do not correlate with the frequency or incidence of breast cancer in developed nations. The milieu of potential carcinogens, promotors, and stimulators to which we are all variably exposed, single or multiple exposure differences and co-carcinogenic risk factors, makes the claim of potential causality in the laboratory difficult to apply to the in vivo experience. These confounding issues may become clear with additional research and investigation, and additional studies to unravel the multiple factors involved in carcinogenesis are needed. However, current scientific knowledge is insufficient to demonstrate a clear cancer risk due to the topical application of cosmetics that contain parabens on normal intact skin. Until scientific study demonstrates otherwise, parabens remain generally recognized as safe. Golden et al[65] claim that "only in vivo data can provide evidence of how hormonally active substances are influenced by absorption, distribution, metabolism and excretion in an intact animal." Consequently, a combined in vitro and in vivo approach is warranted to gain a complete understanding of the biological capabilities of parabens.

Skin Cancer

Topically applied parabens are known to be efficiently hydrolyzed to *p*-hydroxybenzoic acid by cutaneous carboxyesterases, although degradation is not complete.[100,101] The cutaneous enzymes that hydrolyze parabens may be less efficient than previously asserted, with the result being increased risk of persistence in skin and greater absorption.[102] Absorbed parabens that are not degraded in skin are stable in plasma but rapidly hydrolyzed by hepatocyte microsomal esterases, conjugated, and subsequently excreted in urine; the speed of this hydrolysis identified in vitro has led one group of investigators to the conclusion that parabens do not accumulate in human tissue to any significant degree.[103] One study showed 80% to 85% clearance of a single oral dose of different parabens within 24 hours, confirming the efficiency of systemic hydrolysis.[104] If a true reservoir does not exist, repeated application of paraben-containing cosmetics has been implicated in steady-state persistence both cutaneously and systemically.

The possibility that parabens persist in skin and exert measurable biologic effects has been investigated. Ishiwatari et al[23] identified methylparaben unmetabolized and slightly persistent in the stratum corneum after 1 month of daily application. Biological activity identified included altered keratinocyte cellular morphology and decreased proliferating ability; the expression of hyaluronan synthase 1 and 2 mRNAs and type IV collagen was diminished, whereas involucrin and HSP27 (heat shock protein β-1) expression was increased. The authors observed that such changes could impact the aging and differentiation of affected keratinocytes.

The effect of ultraviolet irradiation in combination with parabens on keratinocytes has also been studied. Oxidative stress induction, reactive oxygen species and nitric oxide production, and lipid peroxidation have been reported in vitro, raising the potential to induce keratinocyte carcinomas in patients using cosmetics containing parabens.[25,105] There are no in vivo human studies that identify a role for topically applied parabens in the development of nonmelanoma skin cancer.

The presence of ERs in benign and malignant melanocytic lesions and the variable expression of ERβ over ERα in severely dysplastic nevi and lentigo maligna could suggest that stimulation of these receptors by estrogen and perhaps by exogenous xenoestrogens plays a role in oncogenesis.[106] However, epidemiologic data suggest that women with metastatic melanoma have better survival rates than men, premenopausal women fare better than postmenopausal women, and ERβ expression decreases with increasing Breslow tumor thickness. One study has shown that estradiol increases melanocyte number while decreasing melanin content and tyrosinase activity.[107]

One could hypothesize from these data that topical parabens decrease melanoma risk through xenoestrogenic activity. Much like oncogenesis claims, chemoprotective activity of parabens remains speculative and unsubstantiated and lacks adequate supportive human data.

ANDROGENIC/ANTIANDROGENIC EFFECTS

Parabens have been implicated in the impairment of the reproductive system of male laboratory animals, and concern exists about their impact on human male fertility. Cryptorchidism, reduced semen quality, testicular cancer, and hypospadias are grouped together as part of the testicular dysgenesis syndrome, with parabens suggested as a potential cause due to androgenic receptor action or xenoestrogenic effect.[41] Incidence rate increases of cryptorchidism and reduced semen quality have indeed been reported for the past 10 to 50 years (between 0.8% and 1.4% variance per annum).[108,109] However, a recent detailed review has demonstrated that greater numbers of studies have demonstrated improvement of sperm quality over time than those reporting diminution.[110] Hypospadias frequency has in fact not changed.[111] The grouping of these 4 entities does not necessarily indicate a single identifiable cause.

An in vitro study has shown methylparaben, propylparaben, and butylparaben to be androgen receptor antagonists.[8,41,112,113] The mitochondrion has again been implicated as the potential target organelle in spermatozoa.[114,115] As opposed to estrogens, androgen receptor binding and antiandrogenic activity in parabens are highest with aryloxy side chains (benzylparaben, phenylparaben) and decrease with length of alkyl side chain (weaker consecutively with each CH2 addition 1–4 with no antiandrogen activity for heptylparaben, octylparaben, and dodecylparaben).[116] Antiandrogenic activity of parabens correlated to statistical significance with binding affinity. Ethylparaben and butylparaben have been found to have no endocrine effect on testosterone production, anogenital distance, or testicular histopathology, measured by the H295R steroidogenesis assay, which examines chemicals that affect the production of estrogen and testosterone.[117] Oishi[118–120] found no reproductive hormone function or secretion abnormalities (testosterone, luteinizing hormone, and follicle-stimulating hormone monitoring) in male rats fed 1000 mg/kg per day of methylparaben and ethylparaben, nor were spermatotoxic effects seen for paraben esters or *p*-hydroxybenzoic acid; the same investigator identified butylparaben and ethylparaben to have an inhibitory impact upon serum testosterone, spermatogenesis, and epididymal weights at lower but still relatively high doses of more than 10.4 mg/kg per day. Hoberman and colleagues[121] were unable to confirm these findings, studying methylparaben and butylparaben in exposures up to 10,000 ppm; they postulated that the rapid metabolism of parabens by esterases explained the lack of in vivo effect by these weak estrogens. The Danish EPA confirmed several studies showing young male rats to have adverse effects on sperm production and testosterone levels after oral exposure to parabens, particularly propylparaben and butylparaben; however, other more recent studies with the same study design did not confirm these findings even at very high doses.[6] They further observe that studies exhibiting both positive and negative findings on reproductive toxicity have shortcomings, making it difficult to assess and weigh the results.

It has been stated that these antiandrogenic activities may be due to the xenoestrogenicity of the EDCs rather than the result of specific androgen receptor binding. No human studies have linked the ingestion or percutaneous absorption of parabens to any antiandrogenic physiologic or developmental effect, and the role that parabens and other purported estrogen and androgen receptor antagonists play in vivo remains speculative.

FERTILITY

Fertility rates have decreased progressively in the United States for the past 45 years, and birth rates have decreased in teenagers for the past 15 years.[122] Sperm counts have reportedly decreased by 55% to 60% for the past 40 years according to 1 systematic review and meta-regression analysis.[123] Exposure to parabens and phthalates has been suggested as potentially causative. Ingestion of butylparaben has been implicated in adversely effecting mouse spermatid count and testosterone levels.[124] Conversely, human studies have demonstrated no effect on semen quality parameters or sperm DNA when correlated with urinary paraben excretion in infertility clinic male humans.[125,126] Again, lack of definitive human study data leaves this hypothesis unverified.

PERINATAL EXPOSURE

Very few human studies assessing perinatal exposure to parabens have been performed. Towers and colleagues[127] measured methylparaben levels in the delivery admission and cord blood of 50 mother-child pairs. Forty-seven of each type of sample (94%) showed methylparaben in maternal blood (mean, 20.41 ng/L) and cord blood (mean, 36.54 ng/L). The fetal level was higher than the maternal level in 23 (51%); corresponding figures for butylparaben were lower. These data led the authors to conclude that methylparaben and, to a lesser extent, butylparaben demonstrate transplacental passage.[127] Methylparaben, ethylparaben, and propylparaben esters have been identified in a minority of breast milk samples tested, estimated to be at least 3 orders of magnitude lower than the allowable daily intake of 10 mg/kg of body weight per day.[128,129]

The Vitamin D Antenatal Asthma Reduction Trial measured methylparaben, propylparaben, and triclosan concentrations in maternal plasma samples pooled from the first and third trimesters and in urine samples from children aged 3 to 4 years.[130] Four hundred sixty-seven mother-child pairs were enrolled, and there were no statistically significant associations of maternal plasma or child urine paraben concentrations with asthma or recurrent wheeze or food or environmental sensitization at the age of 3 years; a trend toward an inverse association between antenatal paraben exposure and allergic sensitization was observed.

In a slightly older postpartum cohort, 5 urinary parabens were measured in 436 children in a birth cohort using gas chromatography with tandem mass spectrometry. Methylparaben, ethylparaben,

and propylparaben were the dominant parabens in urinary samples. Positive associations were found between the sum of molar concentrations of 5 parabens and height scores among all children, with statistical significance only observed in boys. The authors concluded that exposure to parabens may be adversely associated with physical growth in 3-year-old boys.[131] These findings lack confirmation to date.

In an animal study, high doses of butylparaben (200 mg/kg of body weight) orally and subcutaneously administered to pregnant rats from gestation day 1 to lactation day 21 revealed social, learning, and memory behavioral deficits in butylparaben offspring in comparison with the controls in addition to certain neurotransmitter abnormalities (monoamine oxidase content along with dopamine, 5-hydroxytrypamine, and γ-aminobutyric acid alterations).[132] Another study was conducted to investigate the potential repeated 28-day dermal toxicity (50, 100, 300, or 600 mg/kg of body weight per day) of IPP, IBP, or a mixture of the two in rats. No significant changes in body and organ weights in any group were found, but histopathologic examination demonstrated mild to moderate epidermal hyperkeratosis in female rats.[133] The lowest observed adverse effect level of the mixture of IPP and IBP was estimated to be 50 mg/kg of body weight per day, with the authors suggesting that the mixture of IPP and IBP exhibited synergistic dermal toxicity with potential implications for future use in consumer products. Investigation using pregnant Sprague-Dawley rats revealed low placental and lactational transfer of dietary butylparaben.[134]

Boberg et al[135] assessed peripartum butylparaben dose effect on the sexual characteristics of Wistar rats. Variable effects depending on dosage (0–500 mg/kg of body weight per day) were found with decreased anogenital distance, reduced ovary weights, and increased mammary gland outgrowth present in exposed female rats. In male offspring, sperm count was reduced at doses greater than 10 mg/kg of body weight per day.

In the murine model, the postweaning exposure to parabens (methylparaben and butylparaben) promoted adipogenesis and suppressed serum marker of bone formation.[136]

PARABENS AND EMOTIONAL SUPPORT

Shiu,[137] evaluating emotional support needs, reported that 20% of American adults require greater amounts of emotional support annually; she characterized these patients as more often middle aged, female, of Latin descent, poor, and with lesser education and a variety of other medical problems. Higher levels of butylparaben, ethylparaben, and methylparaben (along with naphthalene derivatives) were found in their urine; other toxins including heavy metals, arsenic, phenols, phthalates, and pesticides were not associated with these emotional problems.

ANIMAL EXPOSURES TO PARABENS

Investigators in New York determined the presence of elevated levels of methylparaben and the metabolite 4-hydroxybenzoic acid to be among the most abundant chemicals detected in pet food and urine (60 samples).[138] Dry food contained higher levels of parabens and their metabolites than wet food, and cat food had higher paraben concentrations than dog food. The researchers concluded that dogs are exposed to other sources of parabens other than pet food, whereas the predominant exposure in cats is from commercial cat food products.

ENVIRONMENTAL ISSUES

Cosmetic ingredients are potential emerging pollutants, and the environmental monitoring of biocide levels and ecosystem impact is fledgling. With large amounts of parabens used in cosmetic products, particularly moisturizers and sunscreens, it would seem logical that they would receive scrutiny regarding impact on the environment; their biocidal nature could theoretically result in microbe balance disruption. Contamination of surface water with parabens has been claimed to be largely anthropogenic, but there are actually a number of potentially significant natural sources, including blueberries, carrots, olives, and strawberries.[139–141] Natural sources of phytoestrogens may be significant from an exposure as well as ecological standpoint, because they are found in more than 300 foods. The relative binding affinities for the natural phytoestrogens coumestrol, zearalenone, genistein, and daidzein have been assessed to variably bind to ER; avidity was measured between roughly equivalent to parabens to up to 1800 times more avidly bound than various parabens for ERα and from 5 to 12,700 times more avidly bound for ERβ. Estrogen and diethylstilbestrol binding was greater yet (Table 2).[65,142–144] Parabens are also produced in surprisingly high amounts by the marine bacterium of the genus *Microbulbifer* and by the herbaceous plant *Oxalis tuberosa*.[145,146]

The measurement of paraben esters in 9 natural waterways studies from China, Wales, Spain, Switzerland, India, Japan, and the United States has recently been reviewed.[147] Methylparaben was the predominate paraben identified in most studies, ranging from not detected to 1062 mg/L; butylparaben and propylparaben were higher in isolated study regions. The highest paraben found in riverine water was identified in China (propylparaben, 3142 ng/L). Higher paraben concentrations are associated with seasons of low water flow.[148,149]

One would anticipate that parabens in sunscreens would increase the water concentration of parabens, a hypothesis not confirmed by 1 recent Polish study.[150] Conversely, 39 swimming pools in Beijing were examined for 8 common parabens and *p*-hydroxy benzoic acid. Methylparaben and propylparaben predominated at 91.2% of the total parabens, and concentrations detected were roughly 20-fold higher (144 ng/dL) in indoor pools compared with outdoor pools.[151] Considering the total exposure dose of multiple parabens from other sources, the authors assessed human exposure to parabens from swimming pool water as being negligible.

Wastewater treatment plants do not remove parabens and other putative endocrine disruptors totally.[152] The average efficiency of removal of parabens in wastewater treatment plants has been

TABLE 2. Comparative Binding Affinity of 17β-Estradiol, DES, Dietary Phytoestrogens, and Parabens

Compound	Relative ERα Binding Affinity	Relative ERβ Binding Affinity
17β-Estradiol	100	100
Diethylstilbestrol	236	221
Coumestrol	20	140
Zearalenone	7	5
Genistein	4	87
Daidzein	0.1	0.5
Methylparaben	Not calculated	Not calculated
Ethylparaben	0.011	0.011
Propylparaben	0.033	0.044
Butylparaben	0.053	0.123
IPP	0.040	0.054
IBP	0.110	0.093

Modified/adapted from Golden et al,[65] Kuiper et al,[142] Safford et al,[143] and Okubo et al.[144]

DES, diethylstilbestrol.

measured as high, in the range of 96.1% to 99.9%.[153,154] However, parabens were still detected in most of the effluents, at concentrations as high as 4000 ng/L. These and other authors have considered treated wastewater to be a significant potential source of parabens in the environment because of incomplete elimination and the volumes of wastewater processed.[155]

There is limited information available on the ecotoxicological effects of parabens to aquatic organisms. A study performed by the Danish EPA exhibited acute methylparaben toxicity on the green algae *Pseudokirchneriella subcapitata* and the freshwater invertebrate *Daphnia magna*. Benzylparaben (rarely, if ever, used in cosmetics presently because of safety concerns) exhibits the highest toxicity risk, whereas methylparaben and ethylparaben are the least acutely toxic.[156] The Danish EPA evaluated the literature on paraben toxicity to green algae, *D. magna*, and fish, as largely reviewed and published by Yamamoto et al.[6,157] The assessment of fate of these organisms resulting from paraben exposure hazard indicated low toxicity. Estimated risk ratios were calculated as measured environmental concentrations/predicted no effect concentrations of 0.010, 0.0086, and 0.0042 for propylparaben, butylparaben, and methylparaben, respectively. These data indicate a low risk for environmental effect from parabens. Estrogenic effects in fish were noted in concentrations much higher than actual environmental concentrations.

Photo-enhanced toxicity is reported for a number of environmental contaminants including sulfonamides and titanium dioxide nanoparticles.[158,159] A recent study demonstrated that exposure to ultraviolet light magnified methylparaben toxicity to *D. magna*, the most commonly used freshwater invertebrate in standard toxicity testing; the authors were led to conclude that environmental risk assessments based solely on laboratory toxicity data may underestimate toxicity in the natural environment.[160] Unlike benzophenone (oxybenzone), parabens have not been implicated in toxicity, photopotentiated or otherwise, to coral species.

SUMMARY AND CONCLUSIONS

There is a body of in vitro evidence supportive of the weak estrogenic properties of paraben derivatives, along with a large number of other chemical substances. Claims of carcinogenic activity of parabens are less supported by the literature, and no human studies have confirmed significant or even suggestive biological effects regarding hormone disruption, breast cancer, or skin cancer in vivo. The presented data suggest that toxicologic potential seems to increase with side chain length and with benzene ring presence, with methylparaben and ethylparaben rarely implicated. It is the sincere hope of the authors that this review has helped provide perspective regarding what is known and what remains to be determined regarding the toxicology of parabens; the interested reader is referred to a number of excellent review articles that view the subject from different perspectives.[6,14,26,41,64,65,96,143]

Parabens are effective biocides with which consumers, health care professionals, and toxicologists alike have had extensive experience for many decades. Until such time as convincing data are published and verified, claims that parabens have any role in these controversial and important health problems are premature. The questions that have been raised using in vitro and animal investigative techniques await confirmation of any significant biologic effects in humans.

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