

UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE PATENT TRIAL AND APPEAL BOARD

MOM Enterprises, LLC,
Petitioner,

v.

ELAINE AND REINHOLD W. VIETH,
Patent Owner,

Case IPR 2023-00726
Patent 9,066,958

DECLARATION OF REINHOLD VIETH

VIETH EXHIBIT - 2008
MOM Enterprises, LLC, v. ELAINE AND REINHOLD W. VIETH
IPR2023-00726

I, Reinhold Vieth, declare and state as follows under penalty of perjury:

1. I am the co-inventor and one of the owners of U.S. Patent No. 9,066,958 (“the ‘958 Patent”). This declaration is based on personal knowledge unless otherwise indicated.

2. I received my B.Sc. in medical sciences, my M.Sc. in physiology, and my Ph.D. in physiology from the University of Toronto. I also received a post-graduate diploma in clinical chemistry from the University of Toronto. I have authored numerous peer-reviewed and non-peer-reviewed articles during my career. For more than thirty years, I taught at the University of Toronto, retiring in 2012 as a full professor. Attached as Exhibit A is a true and correct copy of my most recent curriculum vitae.

3. I have reviewed the Petition and exhibits filed by Petitioner in IPR2023-00726, particularly the Declaration of Robert O. Williams III, MOM-1004 (“Williams”) and the exhibits to which it refers.

4. Based on my review of the Petition and Williams, MOM-1004, and the exhibits to which it refers, they reflect a fundamental and erroneous misunderstanding regarding “Vigantol” oil and MOM-1006/MOM-1007, (“Harder”), and more critically the problems and issues which the ‘958 Patent addresses and solves.

5. The difficulty posed by dropping one drop of a liquid onto a small surface like a nipple, pacifier, or finger is that much of the drop rolls off. And if that drop is sticky or viscous enough to stay put on that small surface, then it might not be easy to suck or lick from that surface. (If you have vegetable oil on your finger, it will still be there even if you try to lick it off; you will end up washing your hands.) Unless one solves these problems of adherence and delivery, there can be no viable product. The prior art never indicated any awareness of these difficulties and never solved or addressed these technical problems (among others).

6. In addition, one must address the issue of sufficient delivery of an efficacious dosage of the product being delivered. American and Canadian regulations regarding dietary supplements require that for vitamin D, the dose swallowed by the person taking the supplement must be between 90 and 120 percent of the labeled dose. A product or method delivering anything less than 90 percent of the labeled dose is inadequate, unethical and fails to comply with the applicable regulations. During my work on the patent application, I did an extensive background search, looking for objective knowledge about how efficiently a human can lick or suck an oil off a surface. I was very surprised that there was almost nothing to be found in the published literature about this (in terms of library resources, the University of Toronto ranks immediately after Harvard and Yale). The closest pertinent prior that I could find was a Food and Drug

Administration analysis regarding lipstick, which determined that less than 50% of the lipstick composition was actually delivered into the mouth and swallowed. A true and correct copy of this study is attached as Hart, *et al.* (pg. 150 of it), Exhibit B. Hart was considered by the USPTO.

7. None of the prior art disclosed to the examiner or relied on by the Petition discloses the specific compositions and methods of the '958 Patent for use in applying a single drop of vitamin D to an exterior surface of an object so that an infant could suck a pharmaceutically efficacious dose.

8. The Petition's reliance on "Vigantol" oil is misplaced. First, "Vigantol" is simply the name for a particular brand of vitamin D in medium chain triglyceride ("MCT"). Moreover, "Vigantol" for many years was a tablet. It was found to cause toxicity in infants. (Indeed, a significant risk exists that one can overdose infants with vitamin D; this can occur rather easily.) Furthermore, once it became an oil, it was (and is) available only by medical prescription in certain countries in Europe. It is not available in the United States or Canada. One cannot readily obtain a sample, and its actual composition is not readily available or listed on the product packaging.

9. Contrary to the Petition's suggestion, I never seriously studied or worked with "Vigantol" oil. The article I co-authored with Dr. Houghton, MOM-1014, does not suggest otherwise. A review of the reference cited in the context of

the comment regarding the formulation of “Vigantol” in the 1950s, shows that the reference cited discussed “Vigantol” in tablet form, not as an oil.

10. I never used “Vigantol” oil in connection with the research or preparation of the inventions claimed in the ‘958 Patent. For the experimental examples disclosed in the ‘958 Patent specification, I used Miglyol oil, a pharmaceutical quality MCT, and dissolved crystalline vitamin D₃ into it to perform my research. I calibrated vitamin D doses by ultraviolet absorbance and/or liquid chromatography in relation to solutions of vitamin D dissolved in ethanol.

11. I disclosed to the examiner the Holmberg, *et al.* article (“Absorption of a Pharmacological Dose of Vitamin D₃ From Two Different Lipid Vehicles in Man: Comparison of Peanut Oil and a Medium Chain Triglyceride”). This study compared vitamin D bioavailability in MCT versus bioavailability in peanut oil, and determined that peanut oil was better. Holmberg, *et al.* used Miglyol, and they teach away from using MCT to deliver vitamin D, because on an empty stomach, they found that vitamin D prepared in peanut oil was absorbed better than vitamin D prepared in MCT.

12. At column 3 of the ‘958 Patent, beginning at line 27, we disclosed that “a skin lotion that contains vitamin D can be absorbed over time through the skin of the infant.” We pointed out that MCT “are useful as a skin lotion, and at the

same time they can function as a solvent to deliver vitamin D. However, the efficacy of vitamin D delivery is problematic with this approach, because vitamin D could be wiped easily off the skin before it could be absorbed into the body.”

13. We also disclosed to the examiner the Ikushima patent (‘958 Patent, column 3, beginning at line 33), regarding MCT as a solvent for vitamin D compounds. Again, the Ikushima patent teaches away from MCT, since it reports that vitamin D is unstable in MCT that is not pretreated with his method.

14. Furthermore, we disclosed to the examiner the Scanlon, “Vitamin D Expert Panel Meeting – Final Report,” which on its page 21 states without further discussion, “A solution in Germany is to put an oral vitamin D preparation on the mother’s nipple once a day before the baby breastfeeds”. There was no elaboration whether that preparation was aqueous, emulsion, or oil, liquid, powder or cream; there was no mention of how much was to be applied; and there was no mention of what percentage of the applied dose was actually swallowed by the infant.

15. Contrary to Williams’ insinuation, I did not withhold or fail to disclose any relevant or pertinent information or prior art, including “Vigantol” oil, which as I have stated, is simply a brand name for a particular vitamin D product dissolved in an oil. Williams does not appear to have reviewed all the prior art disclosed to the patent examiner, especially the Holmberg and Scanlon references.

Indeed, Williams does not list either of those references in his “List of Materials Considered.”

16. The Petition (and Williams), do not understand, or simply misconstrue, the Harder reference, on which they place so much emphasis. The Petition and Williams’ opinion is premised upon the idea that if you can place a dose of a liquid into the bowl of a spoon, then it is obvious that you can put a single drop of a liquid onto a nipple or pacifier without having it drip off, and still be able to consume at least 90% of that dose by sucking. Their thinking is simply wrong. The Petition (and Williams), are confusing the interior, concave, bowl side of a spoon with the exterior side of a convex surface like a pacifier or mother’s nipple.

17. Harder discusses the conventional use of a spoon. The ‘958 Patent does not deal with the use of a spoon. Harder never states that MCT is uniquely necessary to provide the vitamin D. It just happens that the brand name of the MCT oil she used is “Vigantol”. There certainly is nothing in Harder to suggest that a drop that adheres to the exterior surface of an object, such as a pacifier, a nipple, or another object with characteristics that allow the drop of MCT and Vitamin D to adhere to the object is equivalent to administering a dose of Vitamin D in MCT by use of a spoon.

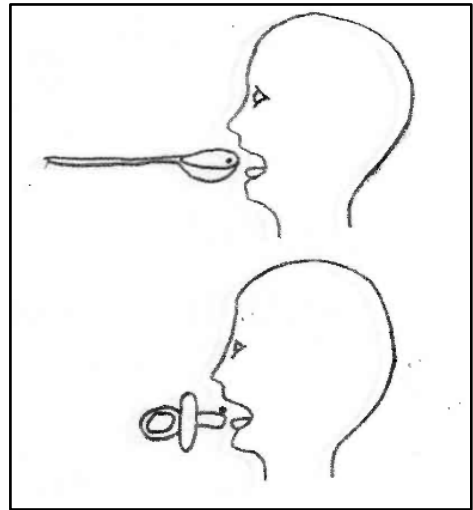
18. Harder never conceived of, did not address, and never solved the problems solved by the '958 Patent. She never showed whether a drop would stick and not drip from a small exterior surface like a pacifier or nipple. Harder used a spoon, which is by definition a long handle with a bowl that holds any liquid. She never addressed the question of how much of the dose was left on the spoon when it was removed from the baby's mouth. She also recognized the significant risk that a spoon has capacity for many drops, and the risk of overdose that posed.

19. Moreover, when a spoon enters a person's mouth, the tongue is underneath the spoon. There is no way to lick a spoon unless it is turned upside down, and that does not make sense in the context of Harder's suggestion. Furthermore, the shape of a spoon is not something that an infant would readily suck on. A spoon is more likely to annoy an infant than stimulate licking. Harder only mentions "lick" and does not mention "suck."

20. The following drawing simplifies, but makes clear, the substantial and significant differences between Harder and the '958 Patent (Harder is illustrated at the top and the '958 Patent is illustrated below).

Figure legend. Methods of giving vitamin D to

an infant. Top, Harder suggestion; Below ‘958 Patent method. The dot represents the location of the dose, which in reality is not a bead, but which spreads as a film coating the surface. Harder places the drop into the bowl of a spoon, which is



not a size or shape that an infant can naturally suck on, and because the baby's tongue is under the spoon, the Harder method makes it impossible to actually lick the dose. In contrast, ‘958 Patent places the drop on the exterior of a size, shape and material that the infant will naturally suck on.

21. Harder thus teaches away from the method claimed in the ‘958 Patent by focusing on an interior, bowl-shaped surface instead of an exterior surface. Harder teaches away from the use of application of one single drop onto a small, convex exterior surface. Harder should be understood to mean to place the vitamin D composition into the concave (interior) bowl of the spoon, because one of ordinary skill in the art would readily understand that medium-chain triglycerides would instantly slide off the back convex (exterior) surface of the spoon and therefore could not be administered in that manner. By contrast, one or more drops can be retained inside the bowl-shaped part of a spoon without concern relating to

adherence or viscosity suggests. Harder presented that water will work as well as a delivery vehicle.

22. The Petition seems to argue either that the interior, bowl side of a spoon is the same thing as the exterior surface of a mother's breast or a pacifier or that somehow the composition (Vigantol oil) will adhere to the back side of the spoon. This basic premise of such an argument is absurd. Harder never validated her suggested approach as one that can deliver the full dose of vitamin D. In fact, much of the dose will remain stuck on the spoon. There should be no doubt that if Harder's suggested approach were widely used, there would be news reports that rickets (a vitamin D deficiency) was occurring despite giving vitamin D to infants with a spoon.

23. Moreover, Harder's use of "Vigantol" oil was an arbitrary choice of a brand name product. The choice had nothing to do with whether or not the liquid could stick to a small surface, whether or not the dose might drip out of the spoon, or whether it was actually removed off the surface of the spoon. Normally, a spoon is used for larger volumes of oil, such as cod-liver oil. It is a normal way to give and take any liquid. For a larger volume, it is obvious that most of the dose is given, because the oily residue that does stay on the spoon represents a small percent of the dose. But a single drop of oil will coat the metal, and after taking a dose, the residue on the spoon is a substantial portion of the intended dose. Harder

never addressed this problem or the problems solved by the ‘958 Patent, and her method has never caught on – probably because it does not work.

24. Harder teaches nothing about the adhering property or any physical aspect of MCT or any other liquids regarding suitability. Harder’s suggestion of putting a dose onto a spoon is absolutely conventional, but that is not what the patent claim says.

25. Furthermore, Harder first suggests dissolving a ground tablet in water and ingesting that from a spoon. (“The tablets disperse quickly in a spoonful of water and can be drunk easily from a spoon.”) Her single sentence, almost passing suggestion of using a single drop of oil, in the context of the risk of overdose, is hardly a disclosure or suggestion of the inventions claimed in the ‘958 Patent, which is a safe and effective method of administering an efficacious dose of vitamin D₃ to an infant.

26. The Petition’s reliance on MOM-1010, Supplement 4.3 of *European Pharmacopoeia* is also misplaced. First, it relates only to MCT and does not mention any active ingredient, Vitamin D or otherwise.

27. Williams relies on MOM-1010 to show the “qualitative and quantitative composition of and the test to be carried out” on medium chain triglycerides. However, this information is impossible to ascertain from the pages of MOM-1010. Many of the characteristics of MCT are stated in terms of

references to standards and tests; however, the standards and test procedures are not contained in MOM-1010. For example, MOM-1010 states that one of the identifications for MCT is that it complies with “B and C”. B states “It complies with the test for saponification value (see Tests).” Under Tests, page 6 of the exhibit states, “Saponification value (2.5.6): 310 to 360.” To interpret this requires knowledge of “2.5.6.” which is not in MOM-1010 and according to the Table of Contents in MOM-1010, not in Supplement 4.3.

28. Williams relies on Harder for the composition of Vigantol oil. In my opinion, it is unreasonable that a person of ordinary skill in the art, which according to Williams, includes a person with a doctorate degree in pharmaceuticals, would look to a baby care book (Harder) for the composition of a prescription-only medication. Williams, MOM-1004, ¶47 relies on Harder MOM-1007, particularly in light of the obvious error in Harder of equating medium-chain triglycerides and “vegetable oil.” One of ordinary skill in the art would know that many vegetable oils are comprised of “long-chain unsaturated fatty acid triglycerides.” Examples given in the ‘958 Patent of such vegetable oils are corn oil and flax-seed oil. MOM-1001, 2:67-3:4.

29. I disagree with Williams that the statement in Harder that Vigantol oil contains only one excipient as a vehicle for the fat-soluble vitamin D, namely, medium-chain triglycerides (vegetable oil). He asserts this means that the oil

necessarily is within the scope of “a composition consisting of ... vitamin D in a liquid triglyceride of 6 to 12 carbon chain length” as recited in claim 1. Williams does not take into account that the ‘958 Patent defines vitamin D narrowly, Ex. MOM-1001, column 3, lines 53-55 and Ex. 2005 (Claim Construction Order). However, Harder does not restrict its definition of vitamin D in the same way as the ‘958 Patent. Further, the Harder description does not exclude components other than vitamin D and “the only excipient as a vehicle for the fat-soluble vitamin D.”

30. The Wolf reference relied on by the Petition lists many liquid vitamin D products, making it clear that there were many liquid options for delivering vitamin D to choose from, most of which were not dissolved in MCT. Wolfe does not reveal anything special about the use of “Vigantol” oil or the use of a spoon.

31. The Blass reference is an example where sucrose was applied to a pacifier so that infants become distracted by the sugar and the sucking, so that they respond less to pain during medical procedures, such as a heel prick. There is no dose or drug involved in Blass. Blass describes this method as “nonpharmacological”, i.e., nothing in Blass is given as a dose.

32. Based on my experience, including attending conferences and giving lectures to audiences that included nurses and midwives, it is my opinion that a nurse does not satisfy the minimum requirements for a person of ordinary skill in

the art pertinent to the '958 Patent. The '958 Patent identifies skills that a person of ordinary skill in the art would have beyond those that an "ordinary nurse" would possess. For example, selecting excipients appropriate for vitamin D, MOM-1001, 1:10-12; understanding the metabolic steps that vitamin D goes through, *id.* 1:40-50; understanding and measuring absorption rates, *id.* at 2:58-66' the skill to understand that efficacious administration of vitamin D must have suitable effects on the circulating concentration of 25-hydroxyvitamin D and how to make that determination, *id.* 4:63-5:7; and others.

33. None of the references relied on by Williams (Harder, Wolf, *European Pharmacopoeia* Supplement 4.3, Blass, and Gardner), either alone or in some combination, teach or suggest the inventions claimed in the '958 Patent. A

person of ordinary skill in the art would not have been motivated to develop the inventions claimed in the '958 Patent in light of those references.

I, Reinhold Vieth declare and state that all statements made herein on my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: June 28, 2023

A handwritten signature in cursive script, appearing to read "R. Vieth".

Reinhold Vieth

Exhibit A

Curriculum Vitae of Reinhold Vieth

Reinhold Vieth, BSc MSc PhD, Certification in Clinical Chemistry, FCACB
 27 Chester Hill Road, Toronto M4K 1X4
 Telephone, 416-469-4772, Cell 416-992-9389

Email reinhold.vieth@utoronto.ca

B. BIOGRAPHICAL INFORMATION

1. Degrees:

DEGREE	UNIVERSITY	DEPARTMENT	YEAR
B.Sc.	University of Toronto	Medical Sciences	1974um
M.Sc.	University of Toronto	Physiology	1975
Ph.D.	University of Toronto	Physiology	1980
Diploma	University of Toronto	Clinical Chemistry	1982

Professional Certifications

Certification by Canadian Society of Clinical Chemists, 1983

Fellow of Canadian Academy of Clinical Biochemists (F.C.A.C.B.*) 1987

*Professional certification indicating scientific and technical competence to oversee the operation of hospital or private laboratory biochemical laboratory service for diagnostic testing of human blood samples.

Distinctions

Canadian Association for Clinical Chemistry Award for Excellence in Research, presented at the CSCC Annual Conference in Quebec City, June 17-20, 2012

2. Employment

Date (# of yrs)	Rank and Position	Department	Institution
2012-ongoing	Retired Professor, and ongoing Adjunct Professor,	Department of Pathobiology and Laboratory Medicine,	University of Toronto
2005-2015	Professor. Cross-appointment,	Dept of Nutritional Sciences	U of Toronto
2005-2012	Professor. Primary departmental appointment,	Dept of Laboratory Medicine and Pathobiology,	Faculty of Medicine, University of Toronto
1990- 2012	Clinical Chemist and Director,	Bone and Mineral Lab	Mount Sinai Hospital
2009- 2012	Clinical Chemist and Director,	Point-of-Care Testing,	Mount Sinai Hospital
2004	Assoc Prof. Cross-appointment,	Dept of Nutritional Sciences	U of Toronto
1999- (4 yrs)	Associate Professor	Pathobiology and Laboratory Medicine	U of Toronto
1985-1999	(14 yrs) Assistant Professor	Pathobiology and Lab Medicine,	U of T
1982-1985	(3 yrs) Lecturer	Clinical Biochemistry	U of Toronto

1982-90 (8 yrs) Laboratory Director Bone and Mineral Group U of Toronto
 1988-1990 (2 yrs) Collaborating Scientist Caribbean Primate Res Ctr U Puerto Rico

Teaching Appointments Held:

2002- ongoing: Course Coordinator sole instructor, LMP1407: Introductory Biostatistics and Clinical Investigation (current 2015 enrollment 120 postgraduate MSc, PhD, clinical chemistry, medical and dentistry residents)

1995 to 2002 (7 yrs) Course Coordinator, LMP1407: From Bench to Bedside: Design, Measurement and Analysis for Clinical Investigators.

2007-10 (3 yrs) Guest lecturer, LMP404: Bone and Skeletal Disorders.

2002 – 2007 (5 yrs) Course Co-Coordinator with Dr W Vogel, LMP404: Bone and Skeletal Disorders.

1995 -Dec/97 (2.5 yrs) Graduate Studies Coordinator Clinical Biochemistry, U of Toronto

1992-1994 (2 yrs) Course co-coordinator with Dr AD Baines, JCL1406: Methods in Clinical Investigation

1995 – 1998 (3 yrs) Course Coordinator, CLB405: Undergraduate Project Course.

1998 - 2002 (5 yrs) Course Co-Coordinator with Dr H Yeger, LMP405: Undergraduate Project Course.

Professional Affiliations and Activities:

Member, American Society for Bone and Mineral Research

Member, Canadian Society of Clinical Chemists

Member, The Endocrine Society

Member, American Society for Nutrition

Journal Reviewer for:

Journal Reviewer In the past year I have served as a peer reviewer of manuscripts submitted to each of the following journals (one manuscript each, including their revisions, unless indicated otherwise)

- American Journal of Clinical Nutrition
- British Journal of Nutrition
- Calcified Tissue International
- European Journal of Pediatrics
- Food and Nutrition
- Journal of Rheumatology
- Journal of the American Geriatric Society
- Journal of Clinical Endocrinology and Metabolism (two)
- New England Journal of Medicine
- Osteoporosis International
- Public Health Nutrition
- Steroid Biochemistry and Molecular Biology

Academic Functions 2012

C. ACADEMIC HISTORY

1. Completed double-blind randomized clinical trial of vitamin D during the weeks leading to radical prostatectomy (I am PI, the first grant application for this work was submitted in 2005, and this has taken 7 years to complete). Our late-breaking abstract was promoted to an oral presentation at the recent American Association for Cancer meeting and was one of only 7 selected for press release. The presentation is available online <http://webcast.aacr.org/console/player/18186?mediaType=slideVideo>
The main achievement of that work was the development by my graduate student, Dennis Wagner, of an assay for calcitriol (the active vitamin D metabolite) in tissue, and it was those tissue calcitriol results that correlated with the key cancer-related outcome changes in the prostate.
2. We published two more papers reporting on our clinical trial of vitamin D in patients with Multiple Sclerosis (see 7, 10 of publications). The trial for which I was co-PI with Paul O'Connor was completed in 2010, , St Michael's Hospital. This work lead Merck-Serono to fully-fund a multicenter clinical trial of our average vitamin D-dosage in MS patients on beta interferon. <http://clinicaltrials.gov/ct2/show/NCT01285401> I am on the organizing group for this trial.
3. Served on two vitamin D panels to advise policy: American Geriatric Society, and Canadian Academy of Health Sciences.

2. Research Awards (Operating Grants)

Year	Individual(s) or Group	Grantor	Amount
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Research Grants:**Held Grants**

2014 Vitamin D Society. Quantification of the effect of Canadian tanning facility practices on the concentration of serum 25-hydroxyvitamin D. \$60,000

2014-2018 Dairy nutrition and risk of diabetes in vulnerable populations: novel insights from biomarkers, measures of marginalization, and their interaction. Dairy Farmers of Canada, Dairy Research Cluster 2 Program. January 1, 2014- March 2018. Investigators: A. Hanley, R. Bazinet, S. Harris, D. Cole, B. Zinman, R. Retnakaran, J. Gittelsohn, S. Bruce, L. Wagenknecht, C. Lorenzo, J. Norris, A. Brown, L. Maple-Brown, **R. Vieth**, C. Lee. Total budget, \$928,000.

2010-2012 Dairy Farmers of Canada. Improved Retention of vitamin D in cheese based on casein-specific binding. 2-yr total, \$246,740.00

2010-2011 Direct-MS. Analysis of samples and data collected in Phase 1-2 clinical trial of high-dose vitamin D in the treatment of multiple sclerosis. \$45,000

2006-2010 National Cancer Institutes of Canada. Vitamin D-dose effects on prostate immunohistochemical markers at prostatectomy, \$129485.00; 2007-8, \$133245.00

Current Research Funding as a Co-Investigator.

Arnaout, Angel (PI) A Phase 2 Randomised, Double-blind, Window of Opportunity Trial Evaluating Clinical and Correlative Effects of Vitamin D in Patients with Breast Cancer (The ICARUS Study) Canadian Breast Cancer Foundation – Ontario Chapter. \$275,050 over 3 year

Tom Wolever PI. Effect of vitamin D supplementation on indices of insulin sensitivity and β -cell function in humans at risk for type 2 diabetes with marginal vitamin D status Dairy Farmers of Canada 3 years \$260,000.

Jonathan Maguire PI, DO IT: Vitamin D Outcomes and Interventions in Toddlers. CIHR Priority Announcement - INMD Startup Funds (Bridge funding) 1 year \$100,000

Dr. Ravi Rajan Retnakaran PI Vitamin D and the Early Pathophysiology of Type 2 Diabetes in Women with a History of Gestational Diabetes CIHR, \$98,000

Raymond Hupperts PI. Sponsor Merck-Serono Merck KgAA Supplementation of Vigantol® Oil Versus Placebo as Add-on in Patients With Relapsing Remitting Multiple Sclerosis Receiving Rebif® Treatment (SOLAR) 3 years Funding at 6 million Euro.

Previous Support

2006-2008 Multiple Sclerosis Foundation of Canada. Vitamin D in predicting risk for and prognosis in multiple sclerosis, \$164300.00; 2007-8, \$112300.00

2006-2007 Dairy Farmers of Canada. DEC Cole, R Vieth. 2006-7, \$68060.00; 2007-8, \$68060.00.

- 2006-2007 Vieth R, O'Connor P. Phase 1B study of vitamin D in patients with multiple sclerosis. \$200,000.00 , DirectMS, Calgary.
- 2006-2007. Clemons M, Cole DEC, Vieth R. Vitamin D as in the palliative care of patients with metastatic breast cancer. \$100,000.00. Vitamin D Society, Research Donation.
- 2004-2006 M Gryn timer, R Vieth, R Kandel. Can vitamin D delay or arrest the progression of Osteoarthritis in an animal model? Canadian Institutes for Health Research. \$83897.00/yr (I am co-applicant)
- 2005-2007 R Vieth, D Rousseau. Bioavailability of Vitamin D from fortified cheese, ice cream and yogurt. Dairy Farmers of Canada, \$38,000 per year. (I am PI)
- 2004-2006 David Cole, Reinhold Vieth. Genetics influences on the variability of response to vitamin D supplementation. Dairy Farmers of Canada. I am co-applicant.
- 2003-2006 Julia Knight, R Vieth. A study of normal light exposure and melatonin in healthy women. \$325,665.00 per year. Canadian Breast Cancer Research Initiative (I am co-investigator)
- 2003-2004 George Ebers. Vitamin D nutritional status as a risk factor for multiple sclerosis: a pilot study. The Multiple Sclerosis Society of Canada. \$50,000. (I am collaborator)
- 2002-2004 Derrick Rousseau and R Vieth. Approaches to the Fortification of Dairy Foods with Vitamin D. Dairy Farmers of Canada, \$38,000 per year. (I am co-applicant)
- 2003-2006 A.M. Cheung, G. Hawker, R. Josse, P. Massicotte, T. Murray, L. Thompson, R. Vieth. Evaluation of the use of vitamin K in postmenopausal women with osteopenia – ECKO trial. CIHR, \$152 563/yr (I am one of 6 coapplicants)
- 2001-2003 El Haj-Fuleihan and R Vieth. Clinical trial of the efficacy of greater vitamin D supplementation for school children in Lebanon. Nestle Foundation, US\$160,000/yr (I am co-applicant)
- 2001-2003 R Vieth, Rubin L, Josse R, Cole DEC. Vitamin D and osteoporosis prevention, healthy postmenopausal women (The POWDER Study). CIHR, Oct 1, 2000 – Sept 30, 2003. \$94,180.00 (I am PI) Funds sufficient to Mar 2005 (subject recruitment was slow, and so was rate of expenditure)
- 2001-2001 R. Vieth. Human Vitamin D Nutrition and its Effects on Gene Expression in Whole Blood. Dairy Farmers of Canada Operating Grant. \$40,000, one-year pilot study (I am PI)
- 2000-2001 R. Vieth. The effects of vitamin D supplementation on immune function of normal adults and responses of patients with metastatic prostate cancer. Centrum Foundation, \$25,000.00. (I am PI)
- 1998-2000 P Walfish, R. Vieth Characterization of vitamin D status in endocrine clinic outpatients. \$15,000/yr. Merck KGaA, Darmstadt, Germany (I am co-PI)
- 1996-97 R.Vieth Ciba-Geigy Canada Operating Grant \$85,000/yr. Renewal, bisphosphonate assays (I am PI)
- 1995-97 R. Vieth Dairy Farmers of Canada Operating Grant \$48,000/yr. D. Cole ; R Vieth was principal investigator. Effects of vitamin D supplementation on normal subjects and kidney stone patients. (I am PI)
- 1995-97 D. Cole , L Rubin, R. Vieth Dairy Farmers of Canada Operating Grant \$83,000/yr. Principal investigator, Dr Cole; Genetics of calcium-sensing receptor (I am collaborator)

- 1994-96 R.Vieth Ciba-Geigy Canada Operating Grant \$60,000/yr. Development of bisphosphonate assays in bone (I am PI)
- 1994 R.Vieth Ciba-Geigy Canada Equipment Grant \$60,000 1992-94 R.Vieth Medical Research Council of Canada. Operating Grant \$58,000/yr. (I am PI)
- 1990-92 R.Vieth Medical Research Council of Canada Operating Grant. \$52003.00/yr. Adaptation dynamics in the vitamin D endocrine system (I am PI)
- 1989-90 S.W. Kooh, Abbott Laboratories, Canada Operating Grant \$25,000 (I am one of two coapplicants)
- 1988-89 R. Vieth Research Institute of the Queen Elizabeth Hospital. \$7773.00 To study the metabolic fate of oral vs intracardial 25(OH)D in rats. (I am PI)
- 1988-89 S.W. Kooh, The Kidney Foundation Operating Grant \$39,800 R. Vieth, J.W. Balfe I was primary author of the resulting paper Publication 14. (I am one of two coapplicants)
- 1987-1988 Kessler MJ, Pritzker KPH, Vieth R. United States Dept. of Health and Human Services. \$5000.00 support for The Caribbean Primate Research Center Program. (I am collaborator)
- 1986-87 R. Vieth. Medical Research Council of Canada. \$10250.00 "Control of 25(OH)D3-24-hydroxylase activity". (I am PI)
- 1984-86 R. Vieth. Medical Research Council of Canada. \$41000.00/yr. "Control of 25(OH)D3-24-hydroxylase activity". (I am PI)
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D. PUBLICATIONS

REFEREED PUBLICATIONS

Published:

- 1: Daley P, Jagannathan V, John KR, Sarojini J, Latha A, Vieth R, Suzana S, Jeyaseelan L, Christopher DJ, Smieja M, Mathai D. Adjunctive vitamin D for treatment of active tuberculosis in India: a randomised, double-blind, placebo-controlled trial. *Lancet Infect Dis.* 2015 May;15(5):528-34. doi: 10.1016/S1473-3099(15)70053-8. Epub 2015 Apr 8. PubMed PMID: 25863562.

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73. Woo TC, Choo R, Jamieson M, Chander S, Vieth R. Pilot Study: Potential Role of Vitamin D (Cholecalciferol) in Patients With PSA Relapse After Definitive Therapy. *Nutr.Cancer* 2005;51:32-6.
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79. Jenkins DJ, Kendall CW, Vidgen E, VIETH R, et al. Effect of high vegetable protein diets on urinary calcium loss in middle-aged men and women. *Eur.J Clin Nutr* 2003;57:376-82. (Coll)
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88. Vieth, R., Cole, D. E., Hawker, G. A., Trang, H. M., & Rubin, L. A. (2001) Wintertime vitamin D insufficiency is common in young Canadian women, and their vitamin D intake does Not Prevent It. *Eur.J Clin Nutr* 55: 1091-1097. (SRA)
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118. Watson AR, Kooh SW, Tam CS, Reilly BJ, Balfe JW and Vieth R (1989) Renal osteodystrophy in children on CAPD: A prospective trial of 1-alpha-hydroxycholecalciferol therapy. Childhood Nephrology and Urology 9:220-227. (Coll)
119. Budden, F.H., Bayley, T.A., Harrison, J.E., Josse, R.G., Murray, T.M., Sturtridge, W.C., Kandel, R., Vieth, R., Strauss, A.L. and Goodwin, S. (1988) The effect of fluoride on bone histology in postmenopausal osteoporosis depends on fluoride absorption and retention. J. Bone and Mineral Res. 3: 127-132. (Coll)
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121. Vieth, R., Fraser, D., Kooh, S.W. (1987) Low dietary calcium reduces 25-hydroxycholecalciferol in plasma. J. Nutrition, 117:914-918. (PA)
122. Kooh, S.W. and Vieth, R. (1980) 25-Hydroxyvitamin D metabolism in the sheep fetus and lamb. Pediatr. Res., 14:360-363. (CPA)
123. Vieth, R. and Fraser, D. (1979) Kinetic behavior of 25-hydroxyvitamin D-1-hydroxylase and -24-hydroxylase in rat kidney mitochondria. J. Biol. Chem., 254:12455-12460. (PA)
124. Vieth, R., Fraser, D., Jones, G. (1978) Modified photography tank for continuous development of thin-layer chromatographic plates. Analytical Chemistry, 50:2150-2152. (PA)

Non-Refereed Publications (not updated)

Invited editorials and commentaries in peer-reviewed journals:

1. Vieth R, Fraser D. Vitamin D insufficiency: no recommended dietary allowance exists for this nutrient. CMAJ. 2002;166:1541-2. (SAR)
2. Vieth, R. AND Carter, G (2001). Difficulties With Vitamin D Nutrition Research: Objective Targets Of Adequacy, And Assays For 25-Hydroxyvitamin D. European Journal Of Clinical Nutrition 55: 221-222. (SRA)

3. Vieth, R. (2001) Reply to J Hathcock and I Munro. Upper intake limit for nutrients. Am J Clin Nutr 74: 866-867. (SRA)
4. Vieth, R. (2001) Reply to FAJ Muskiet et al. Do we really need >100 ug vitamin D daily, and is it safe for all of us? Am J Clin Nutr 74: 862-863. (SRA)

Published Letters to Editor, in peer-reviewed journals:

1. Vieth R, Scragg R. Vitamin D nutrition does not cause peripheral artery disease. Arterioscler Thromb Vasc Biol 2005;25:e41-e42.
2. Barthel, H. R. & VIETH, R. (2004) Lack of generalizable evidence of the superiority of alfacalcidol over plain vitamin D in the treatment of glucocorticoid-induced osteoporosis: comment on the article by Ringe et al. Rheumatol
3. Vieth R. Enzyme kinetics hypothesis to explain the U-shaped curve for prostate cancer vs 25-hydroxyvitamin D in Nordic countries. Int J Cancer, 2003
4. Vieth R. Dairy products, calcium, and prostate cancer risk in the Physicians' Health Study. Am J Clin Nutr 2002;76:490-1.
5. Vieth R, Rao DS. Inaccuracies in relating 25-hydroxyvitamin D to ischemic heart disease. Eur.J.Epidemiol. 2003;18:461-2.
6. Vieth,R. (2000) Problems With Direct 25-Hydroxyvitamin D Assays And The Target Amount Of Vitamin D Nutrition Desirable For Patients With Osteoporosis. Osteoporosis International 2000, 11, Pp. 635-636. (SRA)
7. Peltekova VD, Rubin LA, Uterlinden AG, Hawker G, Vieth R, Trang H, Cole DEC. (1997) . J Bone and Mineral Research. 12:494. R. Vieth was a collaborator
8. Vieth R., Kooh SW, Murray T, Houpt J (1993) Vitamin D supplementation. Can Med Assoc J; 149:396-397. (SRA)
9. Vieth R. (1990) Letter to the editor Re. Alteration of vitamin D metabolism in Mexican-Americans. J Bone and Mineral Research. 5:791. (SRA)
10. Vieth R. (1982) Statistics in "Clinical Biochemistry" papers. Clinical Biochemistry 15: 268

Publications in non-peer-reviewed journals. (Not updated)

1. Optimizing vitamin D supplementation. Vieth R, Taha N. Clinical Laboratory 2011
2. National Post, June National Post Tuesday, June 14, 2005 Page: FP23 Section: Financial Post: Comment Byline: Reinhold Vieth. Financial Post Edition: National Story Type: Business; Opinion: A healthy tan: Excess exposure may be hard on the skin, but the healthful effects of UV outweigh skin cancer and melanoma risks
3. National Post, June National Post Tuesday, June 15, 2005 Page: FP23 Section: Financial Post: Comment Byline: Reinhold Vieth. Financial Post Edition: National Story Type: Business; Opinion: 'D' deprivation: There's no problem with vitamin D intakes to at least 10,000 units per day. But the junkiest science rules with an official safe limit of only 1,000 units

4. OUR VOICE: Living with prostate cancer in Canada (magazine for cancer patients). *Winter 2003, pg12-14*. Sunshine and Supplements: An update on vitamin D. Peter Hyde and Reinhold Vieth
5. R. Vieth. Update on vitamin D nutrition. Whitehall-Robbins Report. Feb 2001 issue (Canadian circulation, 15,000 health professionals)
6. Vieth R. A Review of the Evidence on Vitamin D Supply, Serum 25-Hydroxyvitamin D, and Safety. Bone Depeche, Excerpta Medica, 1999

BOOK CHAPTERS (total, 16)

1. Vieth R. The pharmacology of Vitamin D. in Vitamin D, 3rd edition, edited by D Feldman, JW Pike, J Adams. Eslevier, 2011
2. Vieth R. Unique Features of the Enzyme Kinetics for the Vitamin D System, and the Implications for Cancer Prevention and Therapeutics. D.L. Trump and C.S. Johnson (eds.), Vitamin D and Cancer, Springer Science Business Media, LLC 2010
3. Vieth R. Toxicity of Vitamin D. in Vitamin D: Physiology, Molecular Biology, and Clinical Applications, 2nd edition, editet by Holick MF, Humana Press, 2010.
4. Vieth R, and Sidhom G. Chapter 12. Basic Aspects of Vitamin D Nutrition. In. Contemporary Endocrinology: Osteoporosis: Pathophysiology and Clinical Management. Edited by Robert A. Adler. 2010
5. Vieth R. Chapter: Vitamin D. Encyclopedia of Public Health, 2009
6. Vieth R (2005). The pharmacology of Vitamin D. in Vitamin D, 2nd edition, edited by D Feldman, JW Pike. Eslevier, 2005
7. VIETH,R. (2004) What is the optimal amount of vitamin D for osteoporosis? In: Nutritional Aspects of Osteoporosis (Ed. by P.Burckhardt, R.Heaney & B.Dawson-Hughes). Academic Press, San Diego. Elsevier SRA)
8. Dawson-Hughes, B., Heaney, R., Lips, P., Meunier, P., & VIETH, R. (2004) Vitamin D Round Table. In: Nutritional Aspects of Osteoporosis (Dawson-Hughes, B., Heaney, R., & Burckhardt, P., eds.), Academic Press, New York.
9. R VIETH. Effects of Vitamin D on Bone and the Consequent Evolution of Skin Color. Chapter, in Age Related Bone Loss, an Anthropological Perspective, American Association for Physical Anthropology. (Ed by SC Agarwal and SD Stout) 2003 Kluwer Academic/Plenum Publishers, New York.(SRA)
10. Dawson-Hughes, B., Heaney, R., Lips, P., Meunier, P., & VIETH, R. (2004) Vitamin D Round Table. In: Nutritional Aspects of Osteoporosis (Dawson-Hughes, B., Heaney, R., & Burckhardt, P., eds.), Academic Press, New York.
11. VIETH,R. (2004) What is the optimal amount of vitamin D for osteoporosis? In: Nutritional Aspects of Osteoporosis (Ed. by P.Burckhardt, R.Heaney & B.Dawson-Hughes). Academic Press, San Diego. (SRA)
12. VIETH,R. (2001) Would prehistoric human 25-hydroxyvitamin D concentrations be beneficial, and how much vitamin D do we need to ensure desirable nutritional targets?

- In: Nutritional Aspects of Osteoporosis (Ed. by P.Burckhardt, R.Heaney & B.Dawson-Hughes), pp. 173-195. Academic Press, San Diego. ..(SRA)
13. Vieth R and Cole DEC. (1996) The vitamin D endocrine system. in CRC Handbook of Endocrinology, Volume II, 2nd Edition. Gass and Kaplan, editors. CRC Press, Boca Raton, Florida pp 33-60..(SRA)
 14. Trang H, Cole DEC, Pierratos A, Vieth R (1997) Relative effects of vitamin D2 and vitamin D3 at increasing serum 25-(OH)D pp 727-728. In : Vitamin D: Chemistry, Biology and Clinical Applications of the Steroid Hormone. Printing and Radiographics, University of California, Riverside (SRA).
 15. Vieth R. and Pierratos A. (1991) Urinary calcium and serum 1,25(OH)₂D correlate in kidney stone patients. Eighth Workshop on Vitamin D. pp 907-908. in Vitamin D. Chemical, Biochemical and clinical update. ed AW Norman, M Thomasset and R Bouillon. W de Gruyter, Berlin. (SRA)
 16. Vieth, R. (1988) Evidence that rat kidney 25-hydroxyvitamin D-24-hydroxylase can exist in an inactive state. in Vitamin D. Chemical, Biochemical and clinical update. ed AW Norman. W de Gruyter, Berlin. pp 196-197. (SRA)

PRESENTATIONS AND SPECIAL LECTURES

Podium Presentation co-author: ASBMR, ECTRIMS

CME Activities

Nutrition for Docs. Faculty of Medicine, University of Toronto. Continuing Medical Education (CME) McCloud Auditorium MSB, 2011

- Lecture 1 SUNSHINE VITAMIN D AND HEALTH.
- Lecture 2: Overview of statistical analysis in biomedical research.

Course Coordinator, Faculty representative. Continuing Medical Education Day. Conference title, Diagnosis & Treatment of Vitamin D Deficiency. Nov 3, 2009

Nutrition for Docs. Faculty of Medicine, University of Toronto. Continuing Medical Education (CME) McCloud Auditorium MSB

- Lecture 1. April 26, 2009: SUNSHINE VITAMIN D AND HEALTH.
- Lecture 2: May 30, 2009: Overview of statistical analysis in biomedical research.

Toronto Western Hospital Dermatology Journal Club Speaker Reinhold Vieth, Ph.D., F.C.A.C.B
IMPORTANCE OF THE SKIN TO VITAMIN D And GENERAL HEALTH PLUS:
RELEVANCE OF VITAMIN D TO SKIN. October 28, 2008

Medical Grand Rounds, Sunnybrook Hospital Medical Center, Invited guest presentation: Vitamin D. May 23, 2008

Koffler Student Health Services Professional Staff CME. Vitamin D. University of Toronto, May 23, 2008
Nutrition for Docs, Medical Sciences Building, October 4, 2008. Accredited U of T CME event for physicians.

Mount Sinai Hospital Research Institute. A short overview of biostatistics. June 3, 2008

Princess Margaret Hospital, Urologic Oncology Seminars. Randomized trial of the effects of cholecalciferol on prostate cancer-associated lesions and on cholecalciferol metabolites in prostate tissue April 2, 2008

St Michaels Hospital CME course for physicians. April 4 2008. Reinhold Vieth. Vitamin D – Skeletal and Non-Skeletal Effects SHOULD WE BE ADVISING ALL PATIENTS TO TAKE VITAMIN D SUPPLEMENTS? The “YES” argument Reinhold Vieth

Sponsored professional CME lectures:

Merck: Rheumatology Journal Club, Richmond Hill Ontario, July 16 2008 Octagon Restaurant

Merck: Family Practitioner Journal Club, Windsor Ontario, April 10 2008

Merck: Family Practitioner Journal Club, Kingston Ontario, March 6, 2008

Scarborough Osteoporosis Patient Support Group. Update on vitamin D and osteoporosis. April 28 2008

INTERNATIONAL CONFERENCE PRESENTATIONS

Double-blind randomized clinical trial of vitamin D3 showing effects on tissue calcitriol levels, gene expression and proliferation immunohistochemistry in prostate cancer.

Abstract promoted to oral presentation at international meeting. American Association for Cancer Research April 3, 2012

Three presentations (I) **Optimal nutritional levels of 25 OH Vitamin D: How we measure them, safety and risk of supplementation** (II) **Vitamin D and gynecological cancer.** (III) **Classical And Non-Classical Actions Of Vitamin D.** The conference of Argentinean Society of Gynecological and Reproductive Endocrinology (SAEGRE) Congress, in Buenos Aires city, Argentina, in April 22- 24, 2012 Three separate Invited lectures

Benefits and Risk of Vitamin D Supplements. International Federation of Clinical Chemistry, Bergmeyer Conference, held in Garmish Partenkirchen, Germany March 7, 2012. Invited Lecture.

The Vitamin D Debate: Asserting the Position that “The Institute of Medicine (IOM) guidance for vitamin D is a soothing fairy tale, NOT objective scientific advice to prevent disease” University of Calgary, Department of Kinesiology, Invited expert for academic discussion and debate

Evidence Based Medicine for a Vitamin D RDA when all the evidence is in Shades of Grey. Florida Dietetic Association. Orlando Florida. July 20, 2011. Invited presentation for dietetic professionals

Dosing with vitamin D. National Multiple Sclerosis Society USA. Chicago, USA December 12, 2011. Scientific experts meeting to attempt to design a large-scale trial to “prove” efficacy of vitamin D in preventing Multiple Sclerosis.

Application of Risk-Benefit Analysis in Nutrition Policy: Vitamin D as an example of the policy issues. International Food Technology Conference. New Orleans June 14, 2011 Invited presentation to food-industry professionals.

Dietary Guidelines for Vitamin D and their Implications for Laboratory Testing. Ontario Society of Laboratory Technologists. Annual Meeting, Niagara Falls, Ontario. September 24, 2011. Invited presentation to medical-laboratory professionals.

Minimising the population risk of micronutrient deficiency and overconsumption: The role of food (dietary) supplements. International Alliance of Dietary Supplement Associations. Conference to inform government policy makers from countries in Central and South America. September 1,2 2011 Buenos Aires Argentina. Invited speaker.

Vitamin D safety and toxicity in the context of pregnancy, preterm birth and preeclampsia. Center for Disease Control, Atlanta Georgia USA. April 26 2011. Invited expert for presentation on government information session on vitamin D and pregnancy.

Treatment & Toxicity – Benefits and dangers of supplementing with Vitamin D. Wellcome Trust, London UK. April 7 2011. Workshop sponsored by private trust. Industry-sponsored Workshop Presentation

Vitamin D supplementation during pregnancy & breastfeeding: Effects on gestational diabetes, plus a review of safe and toxic blood serum levels. Wellcome Trust, London UK. May 17. 2011. Workshop sponsored by private trust. Invited Workshop Presentation

Hypovitaminosis D. Medical Grand Rounds, IWK Hospital Halifax Nova Scotia. April 13, 2011

Vitamin D in Pregnancy and Lactation. Nutrition and Dietetics Rounds. IWK Hospital, April 13, 2011

Institute of Medicine Dietary Recommendations in Relation to Serum 25-Hydroxyvitamin D. EuroMedLab Exposition. International Federation of Clinical Chemistry (IFCC) annual conference. May 18, 2011.

Vitamin D and Cancer: U-Shaped-Risk Curves in relation to Serum 25(OH)D. Homburg Germany. R Vieth. International Workshop on Vitamin D and Cancer. Invited workshop lecture.

High-dose oral vitamin D3 administration to prostate cancer patients increases intra-prostate levels of vitamin D metabolites. D Wagner and R Vieth International Workshop on Vitamin D and Cancer. Free communication promoted to oral presentation.

Vitamin D in prevention or treatment of disease. Sioux Lookout regional medical staff of physicians and nurses. Telemedicine Conference. Jan 27, 2011

4th International Vitamin D Symposium on "Vitamin D and Analogs in Cancer Prevention and Therapy" held in Homburg/Saar Germany, May 20/21, 2011. Presenter and sessions Chair.

Euromedlab – International Federation of Clinical Chemistry conference in Berlin (May 18 2011). Discussion of the recently released guidelines from the Institute of Medicine (IOM) and their impact in clinical chemistry practice.

The Wellcome Trust, London, England. Vitamin D during Pregnancy and Lactation. May 16, 2011

Charite Universitätsmedizin Berlin. Berlin, Germany CME for Endocrinologists. Correction of vitamin D deficiency through supplementation. April 9, 2010.

The Wellcome Trust, London, England. Treatment & Toxicity – Benefits and dangers of supplementing with Vitamin D. April 7, 2011

Telemedicine conference Controversies about dietary guidelines and the correction of vitamin D deficiency through supplementation. Sioux Lookout Ontario, physicians and nurses, January 25, 2011

Controversies about dietary guidelines and the correction of vitamin D deficiency through supplementation. University of Rome, Italy, March 28, 2011.

Continuing Medical Education day, Presentation: Hypovitaminosis D. Foggia Italy, March 25, 2011.

Invited expert presenter, "Vitamin D Physiology in Pregnancy: Implications for Preterm Birth and Preeclampsia." Meeting held on April 26-27, 2011 Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, USA.

American Association for Clinical Chemistry (AACC) 2011 Annual Meeting "Vitamin D: Challenges and Opportunities" Atlanta, GA July 27, 2011

Nova Scotia Health, Halifax, Nova Scotia. April 12 and 13, 2011. Lecture to Dietetics Department: The pro's and con's of the latest RDA's for vitamin D.

Grand Rounds, IWK Hospital. Vitamin D and its potential role in pregnancy, and in childhood, and the clinical utility of serum 25D assays, and the question of whether provincial health insurance should continue to fund them for "healthy?" individuals.

Academic debate: The Great Vitamin D Debate. New Guidelines: Agree or not. The Florida Dietetic Association. 76th Annual Symposium July 17-20, 2011

Academic debate: The new Vitamin D Guidelines: Agree or not. University of Calgary, Alberta. Dept of Kinesiology, Markin USRP in Health & Wellness Tuesday, February 8, 2011

Public Broadcasting System (PBS), USA. Panelist on Second Opinion, Topic, Vitamin D, Half-hour, First Episode for this 2011 season. (NB.this is the only program on broadcast television that counts toward CME credits for the American Medical Assoc)

Canadian Society of Clinical Chemists. 14-16 2010 Saskatoon, Saskatchewan. Workshop Presenter: Vitamin D (sponsored by DiaSorin Inc).

- American Association of Clinical Chemistry. Anaheim CA. July 27, 2010 Symposium: 'Vitamin D revisited. Invited speaker Defining Optimal Vitamin D Levels: Reinhold Vieth
- American Association of Clinical Chemistry. Anaheim CA. July 27, 2010 Invited speaker, sponsored by DiaSorin Inc. Invited speaker Reinhold Vieth . Role of the clinical laboratory in vitamin D toxicity.
- Ohio River Regional Chapter of the American Association of Clinical Endocrinologists Annual Meeting July 30-31, 2010 Indianapolis, IN. Invited speaker, Reinhold Vieth. Update on Vitamin D. (2nd time invited in 5 yrs to present to this group)
- Ortho-Clinical Diagnostics, Inc, Rochester NY. June 24, 2010. Invited speaker, Reinhold Vieth. Clinical utility of serum 25-hydroxyvitamin D testing.
- National Institutes of Allergy & Infectious Diseases, Bethesda, MD Conference on IMPACT OF MICRONUTRIENTS ON RESPIRATORY INFECTIONS. May 10-11, 2010. Invited speaker, Reinhold Vieth. Where do we want to be in terms of 25(OH) D, and how do we get there?
- Third International Course on Endocrinology. Bogota, Colombia. April 16, 2010. Invited keynote speaker. Reinhold Vieth. Endocrine aspects of cancer: The role of Vitamin D.
- Ross University Research Day. Medical School Dominica Caribbean. CME May 21, 2010. Reinhold Vieth, guest faculty lecture.
- University of Minnesota. Department of Nutrition. Invited speaker. March 9, 2010. Reinhold Vieth Clinical Nutrition of Vitamin D in the Context of Cancer and Multiple Sclerosis
- 14th Workshop on Vitamin D - Brugge, Belgium, 4 - 8 October 2009. Reinhold Vieth. Podium Debate to defend the position: OPTIMAL HEALTH REQUIRES HIGH 25OHD LEVELS >40NG/ML (>100 NMOL/L).
- Symposium "Vitamin D deficiency, hype or threat" Oktober 2, 2009 , De Eenhoorn, Amersfoort, NL. Reinhold Vieth, keynote speaker. Efficacy and safety of vitamin D3.
- American Association of Clinical Chemistry, Chicago, July 22, 2009. Sponsored lecture, DiaSorin Inc. The Role of the Laboratory in Assessing Concerns Over Vitamin D Toxicity. Reinhold Vieth
- IOM Committee to Review the DRIs for Vitamin D and Calcium, Invited presentation to the committee during its Information Gathering Workshop' held August 4, 2009, in Washington DC. Reinhold Vieth. Safety of Vitamin D
- National Osteoporosis Society, United Kingdom. Annual meeting Manchester, June 29, 2009, Plenary Invited Speaker, Reinhold Vieth. Podium Debate with Dr Roger Francis: optimal requirements for vitamin D.
- Nutrition Society, United Kingdom. Annual Meeting, Surrey, UK. June 30, 2009 Vitamin D: Worldwide insufficiency and effects on health outcomes. Invited lecture Reinhold Vieth.
- RANK FORUM ON VITAMIN D July 2-3 2009 University of Surrey, Guildford. Reinhold Vieth Invited speaker. Optimal 25-hydroxyvitamin D.
- University of Saskatchewan. Edith Simpson Lecture, January 26, 2009. Sponsored lecture, Department of Pharmacy and Nutrition. Wonderful health news about vitamin D, and why the powers that be are not ready to accept it. Reinhold Vieth With followup lecture in the evening at Saskatoon Public Library for the general public.

Public Health Agency of Canada. Invited presentation. March 12, 2009. Reinhold Vieth. The problem of establishing the "proof" that vitamin D prevents multiple diseases.

International Association for Dietary and Supplements Associations. April 22, 2008. Evidence-Based medicine: Are clinical trials the only answer? Reinhold Vieth

Scripps Institute CME: Natural Supplements: An Evidence-Based Update. Lecture title: The Role of Vitamin D and Calcium in Bone Health January 17 - 20, 2008 Paradise Point Resort San Diego, California

Third International Symposium on Vitamin D Analogs in Cancer Prevention and Therapy. Invited Speaker. Optimal Vitamin D for the prevention of Cancer. Krefeld, Germany. **May 17 – 18, 2008**

Invited guest speaker, DSM Human Nutrition Advisory Board Meeting, Basel, Switzerland, Nov 27, 2007

American Association of Clinical Chemists, Sponsored Lecture: Analytical and clinical aspects of vitamin D. Meeting In Washington, July 28-31, 2008

14th Vitamin D Workshop, Bruegge, Belgium. Member of organizing committee. 2008 Planning for October 4-8, 2009.

Brazilian Society for Food and Nutrition October 24-28, 2007, Sao Paulo, Brazil. Keynote Speaker, Reinhold Vieth.

5th International Conference of the European Society of Skin Cancer Prevention, EUROSkin "Reducing the Health Risks of Sunbed Use-Needs for Further European Harmonization of Regulations" 14. – 17. October 2007 Steigenberger Hotel Hamburg Germany. Invited presenter, Reinhold Vieth, "How much vitamin D is needed for health?"

Symposium on Vitamin D in Mother and Neonate. Cleveland, Ohio, October 6, 2007. Invited speaker, Reinhold Vieth, "Vitamin D Requirements in Adults and Toxicity Issues"

NIH Conference on Vitamin D and Health in the 21st Century. Bethesda, Maryland, USA, September 4-5, 2007. Reinhold Vieth, invited speaker, "Potential Adverse Outcomes of Vitamin D"

Center for Bone Health at Childrens Hospital of Philadelphia, Nutrition Seminar Series. May 2, 2007. Reinhold Vieth, Guest speaker, "A fixation on preventing rickets may have led to a neglect of Vitamin D nutrition through the early years of life"

American Academy of Clinical Endocrinology, Ohio River Regional Chapter of "9th Annual Symposium and Business Meeting" August 17-19, 2007, Cincinnati Riverfront Marriott. Invited faculty, Reinhold Vieth, "Vitamin D Deficiency and How to Correct It"

Canadian Laboratory Medicine Congress, Symposium on Biomarkers in Metastatic Bone Disease June 9-13, 2007 Toronto, Ontario, Canada. Reinhold Vieth, invited speaker: "Vitamin D in Cancer – for Diagnosis Only? ... or also for Treatment?"

Merck Latin American Speakers' Forum, Cancun, Mexico, June 1-3 Reinhold Vieth, Invited Faculty, Vitamin D: Its Importance in the Treatment of Osteoporosis

Merck National Osteoporosis Experts Forum, New York NY, May 18, 2007. Reinhold Vieth, presenter and discussion facilitator. Vitamin D: The Potential for Toxicity

National Kidney Foundation, 2007 Spring Clinical Meetings, Orlando Florida, April 11, 2007. Reinhold Vieth, invited faculty, "Role of Vitamin D Nutrition in Health and Renal Disease."

Aurora Health Care, St Luke's Medical Center, Hospital Grand Rounds, Milwaukee, WI, April 5, 2007. Reinhold Vieth, Invited speaker, The spectrum of vitamin D relevance to health and disease in adults.

Novartis, Vitamin D Advisory Board Zürich, Switzerland. 26 January 2007 Invited expert presenter, Reinhold Vieth. Pharmacology of vitamin D.

American Society for Bone and Mineral Research. Vitamin D Toxicity, Policy and Science. Washington, DC. December 4, 2006, available as a webcast

<http://app2.capitalreach.com/esp1204/servlet/tc?cn=asbmr&c=10169&s=20343&e=6950&&>

Tanning World Expo, Nashville, TN. Saturday, October 28 Mid-Conference General Session: Vitamin D and Indoor Tanning. Keynote Speaker, Reinhold Vieth.

New Hampshire Falls Risk Reduction Task Force. Evidence Based Falls Risk Reduction in the Elderly: Taking it Back to Your Community and Making it Work, Phase II. Reinhold Vieth, Vitamin D. June 2, 2006 Mountain Club on Loon, Lincoln, New Hampshire

Hennipen County Medical Center, Department of Medicine, Noon Grand rounds: TITLE: Vitamin D is a pleiotropic nutrient, and adults around the world will benefit from intakes of it that are much higher than current recommendations. November 30, 2006

Henepin County Medical Center, Minneapolis: City-wide endocrine seminars: TITLE: Problems with the way some of the basic principles about the vitamin D endocrine system have been traditionally imagined. November 30, 2006

University of Minnesota, College of Pharmacy. Special Lecturer, graduate program, Natural Medicines Course. Two lectures: 1. Safety and Toxicity of vitamin D. 2. Calcium and Vitamin D controversies. (Host, Professor Rick) February 22, 2006

13th Vitamin D Workshop, Victoria, British Columbia, Canada. Invited Speaker, plus coauthor on two abstracts promoted to oral sessions. April 7 to 12, 2006.

13th Vitamin D Workshop, Victoria, British Columbia, Canada. Member of organizing committee. April 7 to 12, 2006.

House of Commons, London England, Meeting on Sunlight, Vitamin D and Health; organised by Health Research Forum, and sponsored by Ian Gibson MP, Wednesday 2nd November 2005 in the Atlee Room, Portcullis House**

International Workshop on UV Exposure Guidance, Munich, Germany, Joint meeting with WHO and Euroskin; Sun-protection guidelines, implications with vitamin D. October 17-18, 2005.

Direct-MS. Invited speaker: The biology of vitamin D, and its implications for the health of Canadians. Calgary Alberta. Two presentations. October 12 and 13 2005. available as webcast, www.direct-ms.org

Council for Responsible Nutrition. Annual meeting, Palm Springs CA, USA. Invited speaker, "Calcium, Vitamin D and Health". September 26, 2005

Italian Endocrine Society. The endocrinology and nutrition of vitamin D. Invited speaker, Lecture for continuing education. Rome, Italy, July 14, 2005.

30th Public Health Nutrition Update, University of North Carolina. Keynote Lecture: Evaluation of Nutritional Risk for Vitamin D: An Evolving Issue. May 12, 2005

Experimental Biology 2005. R Vieth. Invited speaker for Vitamin D Symposium, to address the topic: Issues and considerations for the safe upper limits of vitamin D intake. San Diego USA April 2-6 2005

Harvard School of Public Health, Boston USA. Jan 28 2005. R Vieth one of 13 experts (the only non US invitee). Symposium called by Dr Walter Willett to request companies to increase the amount of vitamin D in multivitamins and supplements.

National Dairy Council (USA). Vitamin D and PSA levels in men with relapsed prostate cancer. R. Vieth, R Choo, T Woo. Chicago IL, January 13, 2005.

Israel Endocrine Society. The endocrinology and nutrition of vitamin D. Plenary Lecture and one symposium session. December 20-21, 2004, at the Tel Aviv Dan Panorama Hotel & Convention Center.

Cancer Chemoprevention & Cancer Treatment: Is there a role for vitamin D, 1,25(OH)₂-vitamin D₃, or new analogs (deltanoids)? Session II: *Vitamin D, nutrition, and cancer* Session Chairs: R. Bouillon, R. Vieth NIH Campus, Bethesda, Maryland USA November 17-19, 2004.

Harvard School of Public Health, Boston USA. Why vitamin D deficiency will be the “evolving” public health emergency of this decade. R Vieth, Invited Speaker. September 27, 2004.

Wake Forest University, Winston-Salem NC. Grand Rounds, and seminar presentation to Prostate-Cancer Research Group. Mar 17, 2004.

Twelfth Workshop on Vitamin D. July 6-10, 2003, Maastricht, The Netherlands. Debate and Round-Table Discussion: Vitamin D requirements, The High, presented by R. Vieth

5th International Symposium on Nutritional Aspects of Osteoporosis. Presentation, and round-table discussant: “optimal requirements for vitamin D and osteoporosis.” Lausanne/Switzerland, May 14-17, 2003.

32nd Annual Conference, Nutritional Medicine Today. International Society for Orthomolecular Medicine, Toronto, Canada, April 10, 2003

Center for Nutrition Science, Whitehall International. Global Nutrition Advisory Board Meeting, Quebec City, PQ. Sept 30-Oct 2, 2002. Efficacy, safety, and optimal dose of vitamin D for human nutrition.

Joint meeting of the Finnish Society for Nutrition Research and the Finnish Bone Society: Vitamin D – There is more to it than meets the eye. Helsinki, Finland. November 16, 2001. Plenary lecture by R Vieth, Canada: Vitamin D intake and optimal vitamin D status.

Effects of Vitamin D on Bone and the Consequent Evolution of Skin Color. American Association for Physical Anthropology, Kansas City MO, March 28-31, 2001.

4th International Symposium on Nutrition and Osteoporosis, Lausanne, Switzerland, May 17-20, 2000. Invited Lecture. “Estimating the vitamin D requirement and evaluating vitamin D status in patients and populations.” Chapter in the published proceedings, Academic Press, 2001.

First Merck Conference on Life Cycle Management In the Middle East. Cairo, Egypt, May 8-10, 2000. “Rationale for improving vitamin D nutrition in sunny countries, and its relation to calcitriol in the management of osteoporosis.”

The role of vitamin D and the vitamin D receptor polymorphism in patients with idiopathic hypercalciuria. Mineral Metabolism and Endocrine Research Laboratory. University Hospital, Erlangen, Germany. January, 1996.

Local, Ontario presentations

Vitamin D requirements during pregnancy and Onwards: The global evidence. Cleveland Clinic Toronto. December 8, 2011. Invited presentation to professional medical staff.

Sunshine Vitamin D And Health. City-Wide Endocrine Rounds Sept 16, 2011. Academic presentation to endocrinologists from across Toronto.

Sunshine Vitamin D And Health. Presented as accredited continuing medical education at Nutrition for Docs. Medical Sciences Building September 17 2011.

George Brown College, Toronto. Dietitian educational event to discuss the new Recommended Dietary Allowance for Vitamin D. April 5, 2010.

Vitamin D Recent Developments for Prevention in Women . Ontario Medical Association, Women's Health Seminar Apr 29, 2010

Vitamin D – Vitamin or Hormone? Diabetes/Endocrine Update CME Program Chatham, Ontario October 2, 2010

Review of the evidence about vitamin D in prevention or treatment of cancer. Thunder Bay Medical Society CME Thunder Bay Ontario, November 12, 2010.

Crons and Cholitis Foindation. CNIB Conference Centre, Toronto April 25, 2010. Invited speaker to patients and families. Reinhold Vieth, Vitamin D and inflammatory bowel disease.

Cleveland Clinic, Toronto. Wonderful health news about vitamin D, and why the powers that be are not ready to accept it.. Reinhold Vieth. Invited lecture, Feb 12, 2009

Faculty Club, University of Toronto. Continuing Medical Education Day. Conference title, Diagnosis & Treatment of Vitamin D Deficiency. Titles of R Vieth lectures: 9 am Introduction. 2:30pm Solving the D Deficiency--a Safety Profile.

Peterborough General Hospital, Peterborough Ontario. Grand Rounds. Clinical Features of the Vitamin D Story. April 24, 2009

Womens College Hospital, Grand Rounds, September 26, 2007. Reinhold Vieth, The clinical fundamentals of Vitamin D in health and disease.

Womens Health Seminar Series, Centre for Girls' and Women's Health and Physical Activity September 25th, 2006
University of Toronto Athletic Centre, "Sunshine, Vitamin D, Neuromuscular Performance and Health".

June 7, 2006 Osteoporosis Canada summary update for physicians about highlights of the International Osteoporosis Federation Meeting Toronto.

Michener Institute and Osteoporosis Canada Update Course "OSTEOPOROSIS SYMPOSIUM 2006 – MOVING FORWARD" Reinhold Vieth, Vitamin D, November 18, 2006.

PUBLIC LECTURES

Senior Alumni University of Toronto - Canadian Perspectives lecturer series: April 20, 2011: Emmanual College, 75 Queens Park - Room 001 (South Of The Gardiner Museum Of Ceramic Art) TOPIC: VITAMIN D AND OSTEOPOROSIS

University of Toronto Womens' Club Luncheon Lecture. November 16, 2010. Nutrition and Osteoporosis.

Scarborough Osteoporosis Support Group. April **2008-2011**. Vitamin D and osteoporosis. Invited Lecture to approximately 75 lay public

Yonge and St Clair Osteoporosis Patient Support Group annually May, **2007-2011**, organizer, Mrs Debbie Howe

West Toronto Patient Osteoporosis Support Group annually May, **2007-2011** organizer, Mrs Debbie Howe

Oakville Patient Osteoporosis Support Group annually May, **2007-2011** organizer, Mrs Debbie Howe

Osteoporosis Society of Hamilton. May 31, 2007. Reinhold Vieth, Keynote Speaker VITAMIN D: Why do you need it? How much do you need? How best to take it?

Osteoporosis Canada, Bone China Tea, Keynote Speaker: Reinhold Vieth, Vitamin D: Bone and Beyond. Bracebridge, Ontario, May 28, 2007

Yonge and St Clair Osteoporosis Patient Support Group May, 2007, organizer, Mrs Debbie Howe

West Toronto Patient Osteoporosis Support Group May, 2007, organizer, Mrs Debbie Howe

Oakville Patient Osteoporosis Support Group May, 2007, organizer, Mrs Debbie Howe

Scarborough Osteoporosis Support Group. April 23, 2007. Vitamin D and osteoporosis. Invited Lecture to approximately 75 lay public (invited back for 3rd year).

Osteoporosis Canada, Bone China Tea, Keynote Speaker: Nutrition and Osteoporosis, Royal York Hotel, Toronto, November 5, 2006

Yonge and St Clair Osteoporosis Support Group May, 2006

West Toronto Osteoporosis Support Group May, 2006

- Oakville Osteoporosis Support Group May, 2006
- Scarborough Osteoporosis Support Group. April 25, 2006. Vitamin D and osteoporosis. Invited Lecture to approximately 75 lay public (invited back for 2nd year).
- Grand Rounds, St Joseph's Hospital Toronto, "Vitamin D, Victorian vitamin or orphan drug" April 18, 2006.
- Grand Rounds, North York General Hospital, "Vitamin D, Victorian vitamin or orphan drug" April 18, 2006.
- Scarborough Osteoporosis Support Group. April 24, 2006. Vitamin D and osteoporosis. Invited Lecture to the public, Scarborough Village Recreation Center.
- University of Toronto Endocrine Day, November 18, 2005. Invited Speaker, "Osteoporosis fracture risk and vitamin D"
- Nutrition for Docs, Continuing education program, Faculty of Medicine, University of Toronto. Vitamin D and Cancer. November 13, 2005.
- Waterloo, Ontario: Invited presentation for health professionals. November 9, 2005. Update on role of vitamin D in Osteoporosis and General Health.
- Bone and Mineral Group Day, May 27, 2005. University of Toronto. Keynote speaker. Vitamin D and Bone Disease. (this is traditionally for outside invited speakers -- hence, an honour to be a local and invited to speak)
- Scarborough Osteoporosis Support Group. April 25, 2005. Vitamin D and osteoporosis. Invited Lecture to approximately 50 lay public.
- Osteoporosis Update. Vaughn Estates, Sunnybrook Campus. Organized by Dr R Josse, St Michael's Hospital, for continuing education of physicians about osteoporosis. April 1, 2005
- Mount Sinai Hospital. R Vieth: Why you should prefer to be diagnosed with cancer during summer than during winter. April 26 2005, Continuing education sessions for National Medical Technology week.
- Womens Health Matters (an annual show of consumer health products) at Toronto Convention Center. Public lecture, sponsored by Wyeth Pharmaceuticals, R Vieth, Nutrition and Osteoporosis. January 15, 2005, Toronto.
- Mount Sinai Hospital. R Vieth: Nutrition and Osteoporosis. October 28 2004, Continuing education sessions for laboratory technologists.
- Sunnybrook and Womens' College Hospital. Continuing education sessions for laboratory technologists (TML) Noon Rounds, May 12, 2004. Laboratory testing for Osteoporosis, and the clinical nutrition of osteoporosis prevention.
- University Health Network. Continuing education sessions for laboratory technologists (TML) Noon Rounds, April 2003. Laboratory testing for Osteoporosis, and the clinical nutrition of osteoporosis prevention.

Complementary Medicine Physicians' Conference (OMA Workshop) 2004, Invited Speaker, Vitamin D and Nutrition. Bahen Center, University of Toronto, May 29, 2004

Healthy Living Lecture Series: Preventing Osteoporosis. March 19, 2003. Sponsored by Mount Sinai Hospital, Community Relations, Evening lecture, held at Lea Posluns Theatre, North York: audience 500 general public.

Etobicoke Osteoporosis Society, Vitamin D and Osteoporosis. April 5, 2003

University Health Network. Continuing education sessions for laboratory technologists (TML) Noon Rounds, April 2003. Laboratory testing for Osteoporosis, and the clinical nutrition of osteoporosis prevention.

Citywide Rheumatology Rounds. Bone markers, the science and what we have available for the clinician. Hospital for Sick Children, Dec 17, 2002

Mount Sinai Hospital. Endocrinology Rounds. March 10, 2002

University Health Network. Osteoporosis Noon Rounds, February 16, 2001. The story of vitamin D nutrition and osteoporosis.

Ontario Association of Histotechnologists, The Michner Institute, Toronto, November 23, 2000. "Vitamin D: Its effects on health beyond just the bones."

The Hospital for Sick Children, Toronto, Seminar Series. October 2, 2000. Calcium and Bone Metabolism. R. Vieth.

Pathology and Laboratory Medicine, Mount Sinai Hospital, Continuing Education for Technical Staff. "Vitamin D Nutrition and Osteoporosis" September 6, 2000.

Evidence that current recommended vitamin D intakes are too low for adults. R. Vieth. Connective Tissue Research Group, Mount Sinai Hospital, Toronto. Monday April 12, 1999.

Bone markers relevant to osteoporosis.. Toronto Society for Clinical Chemistry. November 8, 1995.

Role of the vitamin D endocrine system in kidney-stone patients. R.Vieth and D.Cole. Grand Rounds Lecture, The Wellesley Hospital, Toronto, June 14, 1995.

Collagen crosslinking of the cartilagenous matrix: in vivo and in vitro. H Gahunia, R Vieth, KPH Pritzker. Inaugural Conference of Canadian Musculoskeletal Society, Montreal, June, 1995.

Responses in vitamin D-metabolizing enzymes to altered nutritional status. R Vieth. Department of Paediatrics, McMaster University, February 7, 1994.

New biochemical markers of bone resorption. City-Wide Rheumatology Rounds, Toronto. January 5, 1993.

Why is the endocrine regulation of calcitriol so complicated? Departmental Seminars. Department of Clinical Biochemistry, University of Toronto. February 5, 1992.

Unique aspects of the hormonal control of vitamin D metabolism.. Departmental Seminars. Department of Clinical Biochemistry, University of Toronto. April 4, 1989.

National, Canadian presentations

Halifax: The Bone Club. Vitamin D and Bone Health. November 8, 2006.

Halifax: Endocrinology Rounds. Vitamin D and Endocrine Aspects of Bone Health. November 9, 2006.

Moncton NB, Pharmacy Refresher Course, Merck Satellite Symposium, Vitamin D. October 14th 5:00pm- 6:30pm. Crowne Plaza Hotel.

BC Dairy Foundation, 7th Annual Milk and Cookies Event, "Vitamin D – Are We Getting Enough?" June 26, 2006. Vancouver BC.

Canadian Cancer Society: North American Conference on UV, Vitamin D and Health. Metropolitan Hotel, March 8, 2006, Toronto.

Direct-MS, Calgary. Presentation to health professionals in Calgary, Alberta. October 12, 2005. Prospects For Vitamin D Nutrition For Disease Prevention And Treatment.

Direct-MS, Calgary. Health information presentation to the general public. An advertised lecture, in Calgary, Alberta. October 13, 2005. Health Prospects For Vitamin D Nutrition. Recorded and available as a Webcast <http://www.direct-ms.org/presentations.html>

Quebec Society of Medical Biochemists. Mont Saint Hiliard, Quebec. October 27, 2005

Merck Frosst Canada. Scientific/Medical Associates. Toronto. Vitamin D and Osteoporosis. January 26, 2005

University of Ottawa, Osteoporosis Rounds. Invited speaker, host Dr John Gay. Clinical applications of vitamin D. April 15, 2005

Wyeth Pharmaceuticals, R Vieth, Keynote Speaker, Nutrition and Osteoporosis. Re Canadian Product Launch of *Caltrate Select*, a new osteoporosis-prevention supplement. Mount Sinai Hospital, October 28 2004, Toronto.

Merck Frosst, Canada. Meeting of Medical Advisory Committee for Musculoskeletal Health. R. Vieth to speak on "Vitamin D and Osteoporosis". Toronto, October 22, 2004

Annual nutrition workshop, Nutritionists of the Ontario Public Health Association. Niagara on the Lake, May 27, 2004. Update on vitamin D and health.

Dietitians of Canada, Dairy Farmers of Canada, Montreal, Sept 23, 2003 Review of the role of vitamin D in the health of Canadians.

Annual meeting of Nutrition Associates, Dairy Farmers of Canada, Banf, Canada, May 15, 2002. Vitamin D and health.

Grand rounds, two different lectures: Jewish General Hospital Montreal, Quebec. May 15, 2002; Royal Victoria Hospital, Montreal Quebec. May 16, 2002

Plenary Lecture: Diagnosis and consequence of what may be, by objective means, our most common "nutritional" deficiency -- vitamin D. Upstate New York Section, American Academy of Clinical Chemistry. Royal Connaught Hotel, Hamilton, Ontario. May 5-6, 1998.

Biochemical markers relevant to osteoporosis. Telemedicine Canada Speaker- Broadcast Presentation. Faculty of Medicine, University of Toronto. February, 20, 1996.

Biochemistry of Vitamin D. Telemedicine Canada Speaker- Broadcast Presentation. Faculty of Medicine, University of Toronto. February, 27, 1996.

E2. PAPERS PRESENTED AT MEETINGS.

Human colon and serum concentrations of 1,25-dihydroxyvitamin D are positively correlated
Wagner, D. Dias, A. van der Kwast, T. **Vieth, R.** Ameican Society for Bone and Mineral Research, Toronto, Canada October, 2010.

R. Vieth, R. Choo, L. Klotz, G. Deboer, C. Danjoux, and G.C. Morton. Seasonal variation in the rate of rise in prostate-specific antigen in patients with untreated, clinically localized, low-to-intermediate grade, prostate carcinoma. Presented at: Cancer Chemoprevention & Cancer Treatment: Is there a role for vitamin D, 1,25(OH)₂-vitamin D3, or new analogs (deltanoids)? NIH Campus, Bethesda, Maryland USA November 17-19, 2004

T.C.S. Woo, R. Choo, M. Jamieson, R. **Vieth**. Vitamin D (cholecalciferol) slows the rise in prostate specific antigen in men with relapsed prostate cancer T.C.S. Woo, R. Choo, M. Jamieson, R. Vieth. Presented at: Cancer Chemoprevention & Cancer Treatment: Is there a role for vitamin D, 1,25(OH)₂-vitamin D3, or new analogs (deltanoids)? NIH Campus, Bethesda, Maryland USA November 17-19, 2004

T.C.S. Woo, R. Choo, M. Jamieson, R. **Vieth**. Vitamin D (cholecalciferol) slows the rise in prostate specific antigen in men with relapsed prostate cancer T.C.S. Woo, R. Choo, M. Jamieson, R. Vieth. Presented at: Cancer Chemoprevention & Cancer Treatment: Is there a role for vitamin D, 1,25(OH)₂-vitamin D3, or new analogs (deltanoids)? NIH Campus, Bethesda, Maryland USA November 17-19, 2004

G. El-Hajj Fuleihan¹, A. Arabi¹, M. Choucair^{*2}, M. Nabulsi^{*3}, J. Maalouf^{*1}, **R. Vieth**,. Effect of vitamin D supplementation on bone gain of adolescent girls. American Society for Bone and Mineral Research Adolescents American Society for Bone and Mineral Research, Seattle WA USA Oct 2004 oral presentation.

A. Arabi¹, M. Choucair^{*2}, M. Nabulsi^{*3}, J. Maalouf^{*1}, **R. Vieth**, G. El-Hajj Fuleihan¹. Bone Mineral Density by Age, Gender and Pubertal Stages in Healthy Lebanese Children and Adolescents. American Society for Bone and Mineral Research Adolescents American Society for Bone and Mineral Research, Minneapolis, WI USA Sept 19-23, 2003

J. Maalouf^{*1}, L. Zahed^{*2}, **R. Vieth**, M. Nabulsi^{*4}, M. Choucair^{*5}, G. El-Hajj Fuleihan¹ VDR Polymorphisms and Musculo-Skeletal Parameters in Lebanese Adolescents American Society for Bone and Mineral Research, Minneapolis, WI USA Sept 19-23, 2003

Vieth R. Why it is important to stop the erroneous practice of referring to vitamin D as a hormone. 12th Workshop on Vitamin D. Maastricht, The Netherlands, July 6-10, 2003

Vieth, R., Dogan, M., Cole, D. E., Rubin, A., Bromberg, I. L., Josse, R., and Walfish, P. G. Vitamin D3 at 90 or 7000 mcg weekly for 1 year: Responses of 25-hydroxyvitamin D, PTH, urine and plasma calcium. 30th European Symposium on Calcified Tissues, Rome, Italy. May 8-12, 2003

Vieth R, Walfish PG., How Much Vitamin D Supplementation do Adults Need to Ensure a Serum 25(OH)D Concentration Optimal for Suppression of PTH? American Society for Bone and Mineral Research, Toronto, Ontario, 2000. Cited for Plenary Award Session.

VIETH,R., CHAN,P.C. & MACFARLANE,G.D. (2000) Randomized, prospective evaluation of effectiveness and safety of vitamin D3 intake exceeding the lowest observed adverse effect level. *The Journal of Bone and Mineral Research*, 14, pp. S538.

David E. C. Cole, Hoang M. Trang, Reinhold Vieth, Vanya D. Peltekova, Andreas Pierratos, Betty Y.-L. Wong, Laurence A. Rubin, Geoffrey N. Hendy. Calcium Excretion is Independently Associated with the Vitamin D Receptor (VDR) and Calcium-sensing Receptor (CASR) Polymorphisms in a Nephrolithiasis Population. American Society for Bone and Mineral Research, San Francisco, December 1-6, 1998. Plenary Poster Sessions Award.

PATEL,M., COLE,D.E., SMITH JC, WONG B, TRANG,H., VIETH,R., MELZER P, HAWKER,G. & RUBIN,L.A. (1999) Alleles of the estrogen receptor alpha gene and an estrogen receptor cotranscriptional activator gene, amplified in breast cancer-1, associate with quantitative calcaneal ultrasound and femoral neck bone mineral density. *Journal of Bone and Mineral Research*, 14, pp. meeting presentation.

R. Vieth, L. A. Rubin, H. M. Trang*, G. A. Hawker, D. E. C. Cole. The Prevalence of Marginal Vitamin D Deficiency Increases Every Winter in Healthy Young Toronto Women. American Society for Bone and Mineral Research, San Francisco, December 1-6, 1998.

Trang H, Cole DEC, Vieth R. Relative effects of vitamin D2 and Vitamin D3 at increasing serum 25(OH)D, Tenth workshop on Vitamin D, Strasbourg, France, May 24-29, 1997. Abstract p.185.

Trang H, Rubin L, Cole DEC, Pierratos A, Vieth R. Comparison of vitamin D nutrition in normals and kidney stone formers. . American Society for Bone and Mineral Research, Cincinnati, September 10-14, 1997.

Cole DEC, Peltekova VD, Rubin LA, Hawker GN, Vieth R, Liew CC, Hwang D, Evrovski J, Hendy G. Genetic determinants of serum calcium: Potential roles for A986S and R990G polymorphisms of the calcium-sensing receptor (CASR) gene. . American Society for Bone and Mineral Research, Cincinnati, September 10-14, 1997.

Cole DEC, Kooh SW, Binet A, Vieth R. Primary infantile hypomagnesemia in a 22-year old man: daily parenteral magnesium injections can be replaced by; nocturnal nasogastric infusions but not by high-dose calcitriol. Canadian College of Physicians and Surgeons, 1996

Cole DEC, Rubin LA, Hawker GA, Ragheb R, Vieth R, Brunet J-S. Effects of population structure on vitamin D receptor (VDR) polymorphism frequencies and their implications for genetic analysis of metabolic bone disease. American Society for Bone and Mineral Research, Seattle, September 6-11, 1996.

Gahunia HK, Vieth R, Pritzker, PH Pritzker. Collagen crosslinking of the cartilaginous matrix: in vivo and in vitro. Inaugural Canadian conference on the musculoskeletal system. Montreal, summer 1995.

Cheng PT, Aye LM, Vieth R, Muller K. Cyclical parathyroid hormone and estrogen treatment of osteopenic ovariectomized adult rats. American Society for Bone and Mineral Research, Baltimore MD, Sept 9-13, 1995.

Cheng PT, Aye LM, Vieth R, Muller K. Higher estrogen dose is required in cyclical parathyroid hormone and estrogen treatment of osteopenic ovariectomized rats than in preventive treatment with estrogen alone. International Conference on Animal Models in the Prevention and Treatment of Osteopenia. Cairns, Australia, February 11-13, 1995

E3. MEDIA COVERAGE/INTERVIEWS RELATED TO SCHOLARLY ACTIVITIES

October 22, 2015. CBC Television, Nature of Things Documentary: The Curious Case of Vitamins and Me.

December 17, 2014 CTV News. Interview regarding: Vitamin D supplement does little to reduce heart disease, stroke rates: study.

Mount Sinai Foundation 2011. Informational internet video:

Vitamin D - Q&A with Dr. Reinhold Vieth

<http://www.youtube.com/user/MountSinaiFoundation#p/a/u/0/H5HiBtY3ccw>

University of Toronto Magazine Spring 2010. The Vitamin D Crisis A full Interview with Reinhold Vieth. Posted By Scott Anderson On March 16, 2010

Financial Times Magazine, UK and International editions. Weekend magazine, and in its international edition, October 23, 2009. Cover story: profile story about vitamin D with a focus on researcher Reinhold Vieth.

SCHRÖDINGERS KATT - NRK TV, Science Dept. Norwegian Broadcasting Corporation Interview with Reinhold Vieth about artificial tanning and health. Broadcast, January 2010

CBC, CTV, and Global Television interviews, March 24, 2010 about Statistics Canada publication of Canada-wide results for serum 25(OH)D levels. On-camera news items on CBC National News, CTV National News, Global TV News, CBC Radio Toronto as well.

BBC Radio 4. The Food Programme Aug 2 2010. Vitamin D dietary guidelines, full episode available at <http://www.bbc.co.uk/programmes/b00t66nr>

American Association for Clinical Chemistry (AACC) Clinical Laboratory News, July 2009. Interviewed for cover-page article about analytical issues of vitamin D lab testing.

CBC Radio Quirks and Quarks June 7 2008. Vitamin D and human evolution.

Business News Network (BNN). June 2007. Cancer and vitamin D interview

CBC Radio One, June 7 2007. Fourteen separately scheduled live interviews from CBC affiliates across Canada Regarding the new Canadian Cancer Society advice that all adult Canadians should "consider" supplementing with 1000 IU vitamin D daily.

Canada AM, CTV Network. January 2007. Interview about Vitamin D and cancer. R Vieth.

MORE Magazine. Who's afraid of vitamin D? Jacqueline Hennessey. Summer issue 2007.

Homemaker's Magazine, May 2006. in article, "How to build better bones."

Good Housekeeping, March 2006. in article, "Vitamins that you really need".

Readers Digest, Canada, March 2006, pg 83; Building Better Bones

Toronto Star, Vitamin D feature article, Feb 24, 2006

Ottawa Citizen, Vitamin D feature article, Jan 2006

Newsweek Magazine. Interviewed for feature story, for early January, 2006

Discovery Health Channel. Medical Hotseat, First broadcast Aug 21, 2005: Sun Safety – a panel debate. One of 4 panelists.

CTV News. January 15/16 2005. Story about Vitamin D and cancer. R Vieth.

Newsweek Magazine. Interviewed for feature story Jan or Feb 2005.

Hamilton Spectator. Feature story on vitamin D (different from below). December 6, 2004

Winnipeg Free Press. Feature story, Vitamin D. November 8, 2004

London Free Press (and Canoe website). November 6, 2004.

CITY TV/Pulse24. Vitamin D, sunshine story. October 20, 2004. Laura DiBaptista.

Readers Digest, October 2004 cover story. The Healing Vitamin: Even with a healthy diet, you may be D-ficient. By Diane Peters

Science News. The weekly newsmagazine of science. October 16, 2004. Vitamin D: What's enough? Feature story by Janet Raloff – one of several experts interviewed.

Journal of American Medical Association. September 22/29, 2004—Vol 292:1416. Scientists Probe Role of Vitamin D: Deficiency a Significant Problem, Experts Say Catherine Meyer, MD

WebMD. Vitamin D may ease depression. Sid Kirchheimer. August 2, 2004

Medical Post, Canada. Skin wars: Dermatologists warn about cancer while nutrition experts bemoan vitamin D deficiencies. Who's right in the battle under the sun? Celia Milne. July 28 2004

CMAJ, May 2004. Interviewed for opinion regarding academic freedom.

The Scientist, April 19, 2004. Interviewed for opinion regarding academic freedom.

Kansas City Star. April 6, 2004. Vitamin D-ficiency Recent studies suggest we aren't getting enough of a good thing. By Karen Uhlenhuth, Syndicated throughout the US.

AMED News (weekly newspaper of the American Medical Association) April 5, 2004. Vitamin D found to boost health, if people get enough: Recommendations for increasing intake of the nutrient are under consideration and sometimes trigger controversy. <http://www.ama-assn.org/amednews/2004/04/05/hlsb0405.htm>

Canadian Living Magazine for issue of April 2004: New advice on vitamin D intakes. By Fran Berkoff

CBC Television, The National. Vitamin D and multiple sclerosis. January 12, 2004

Canadian Press. Vitamin D and multiple sclerosis. January 12, 2004

National Post Newspaper Vitamin D and multiple sclerosis. January 12, 2004

Canadian Press, and the Toronto Star. Jan 20 2003 More vitamin D may help seniors fight osteoporosis, researchers say.

Good Times Magazine, November, 2002. Interview and article, vitamin D and osteoporosis/ the POWDER Study

Canadian Living Magazine, November, 2002. Interview and article, vitamin D and osteoporosis/ the POWDER Study

Medical Post of Canada. January 15, 2002 Women need more vitamin D: Official recommended levels offer no benefit. David Hodges.

Reuter's Health News Service. December 11, 2001

The Globe and Mail. November 20, 2001 The ABC's of Vitamin D. Its other health effects, and potential role in multiple sclerosis. Avis Favaro.

CTV National News, November 18, 2001. Vitamin D intake is not enough to have much effect on adults.

Full, 1-hr Radio interview: Your Own Health and Fitness, KPFA 94.1 FM, San Francisco. Layla Barman: How much vitamin D do we need? November 28, 2000; repeat broadcast, Jan 2003

Your Baby Magazine, Interview and article, May 2000.

Clinical Pearls News Interview, 1999, and feature article.

Canadian Living Magazine, June, 2000. Vitamin D nutrition

Talk 640 Radio, Healthy Maturity – 30-min on air interview, June 20, 1999

CHFI Radio, Chronicle, Toronto, January 18, 1999

CBC Radio, Whitehorse, Yukon, January 12, 1999

CBC Radio, Fredericton, New Brunswick, January 7, 1999

The Weather Channel, Chris McDermott. Vitamin D deficiency is common in many Canadians in winter. Telecast at several times daily in their cycle of weather related stories, over 2 week span, January 1999

Toronto Sun, and across the Sun Newspaper chain – a separate story from the above, Marlene Habib, Winter and vitamin D, January 18, 1999

Canadian Press, Marlene Habib, Vitamin D deficiency is common in many Canadians in winter. Ran in many Canadian daily newspapers, including London Free Press, and Canoe.com Sun newspaper chain website, December 23, 1998

CITY TV News (Laura DiBatista), vitamin D deficiency in Canadians. April 14, 1998

CTV NewsOne, April 14, 1998

CTV National News (Avis Favro), April 13, 1998

The Toronto Star, and other Canadian papers, Vitamin D for winter in Canada. Front page of Life section feature article, January 2, 1998

F. TEACHING AND DESIGN

Courses for which I was or am Co-ordinator (responsible for design and operation of course):

LMP1407, 1991 to present (initially coded as course JCL). School of Graduate Studies. This winter-term course was initiated in 1991 by Dr Baines and myself to provide clinical research skills for our graduate students and residents. I have been the primary coordinator for this course since 1993; and sole lecturer since 2002, teaching 26 hrs of teaching biostatistics.

INNOVATION: These classes are held in the Computer Lab of Medical Sciences Building; the "text" is SPSS statistical software, and the teaching focuses on the principles of statistics needed to use that. Current enrollment, 120 postgraduates.

Consistently, retake rate >90%, see student comments; current enrollment 61 post-graduate students, most students in the course are from departments outside LMP. This course is a requirement for postdoctoral clinical chemistry diploma residents.

LMP404. Started Sept 2002, ongoing. Bone and skeletal disorders. INNOVATION: This was a completely new Faculty of Arts and Science course to support LMP's undergraduate specialist's program. In the first year of this course I designed every aspect of the course, and provided 10 hrs of lectures. Further, for guest lecturers who did not provide study notes suitable the students, I reworked the material so there would be handouts. In addition, there was a web page with all notes and discussion group for the students. 35% of the mark was based on presentation and handout done by the students themselves. Overall, this was a very successful premiere for a course. This year, Dr W Vogel will be sharing these responsibilities.

LMP 405. 1995 to 2002-3 (formerly CLB405). Faculty of Arts and Science. I have been co-ordinator of undergraduate independent research courses for department of Laboratory Medicine and Pathobiology or Clinical Biochemistry for 8 yrs; in recent years. This involved 1 initial hour to introduce the course and its objectives, ongoing communication with students, and arranging the process of examination and marking of laboratory reports.

G. GRADUATE STUDENT THESIS SUPERVISION

Current PhD Students: One:

Dennis Wagner (Dept Nutritional Sciences)

Current MSc Students: Two

Banaz Al-Khalidi (Dept Nutritional Sciences)

Nadeen Taha (Dept Nutritional Sciences)

During career, have been supervisor of the following:

(Successfully Completed)

A. DOCTORAL

Samantha Kimball (PhD, 2011, Dept of Nutritional Sciences)

Heather Hanwell (PhD 2011, Dept of Nutritional Sciences)

Student Harpal Gahunia. (cosupervision with Dr Pritzker)

B. MASTER'S Student Hoang Trang (Primary supervisor, Dr Cole was cosupervisor)

C. POST-DOCTORAL FELLOWS Dr Lindsay King, and Dr Irina Piatkov

Member of Supervisory Committees

Doctor of Philosophy:

Current:

Andrea Glenn, NFS Graduate (MSc) Committee

Raha Jahani, NFS Graduate (MSc) Committee

Christina Fielding, NFS Graduate (MSc) Committee

Holly Pang Pharmacy Graduate (MSc) Committee

Edwin Chow Pharmacy Graduate (PhD) Committee

Doris Li, LMP Summer Student Project Supervisor

Margot Latanzi , NFS Undergraduate Project Supervisor

Previous

Wei Deng, Dr J Heersche

Rachel Zand, supervisor, Dr EP Diamandis

Wei Li, 1998-2000, supervisor, Dr J Heersche

Nostrad Zarmagi., 1997, Dr. EP Diamandis

He Yu, 1996. supervisor, Dr. EP Diamandis

Gail Anderson, 1996 (internal referee). supervisor, Dr. J. Heersche

Xiauyen Wu, 1996 (internal referee) supervisor, Dr. H. Sonnenberg

Lola Bartoszewicz, 1995. supervisor, Dr. F. Keeley

Amin Wang, 1995. supervisor, Dr. D. Templeton

Master of Science:

Sujatha Chakravarthi (ongoing), Dr Venket Rao

Michael Lee, 2002, supervisor, Dr. CC Liew

Wei Ding, 1999, supervisor, Dr. D. Templeton

DOCUMENTATION OF TEACHING

COURSES TAUGHT

a) UNDERGRADUATE

MEDICINE

classroom hours	PBL tutor	seminar leader
1996-97	4	2
1997-98	2	
1998-99	2	
1999-present		4 hours (annually)

I was a PBL tutor for 3 yrs, and have been a seminar leader in the Meds Nutrition and Metabolism course for most years since 1982

ARTS AND SCIENCE

1996.2002 Lectures, 2 hrs each year for course NFS488H, Food Safety and Toxicology, Department of Nutrition, Dr Vinket Rao, course coordinator

1996-2003 LMP405 Undergraduate project course. Coordinator of the course, and each year supervised at least one student.

2002-2005 LMP404 LMP404h Skeletal Disorders. Coordinator of the course. Teach 6h/term, and oversee student presentations

2003-2004 Anthropology guest lecturer, nutrition and evolution.

2002-ONGOING NFS487H Nutrigenomics 2hrs

PHARMACY

1996-present 2 hours each year, statistics and the interperetation of clinical laboratory data.

2010-2012 One hour each year, calcium metabolism, bone and mineral and the laboratory

b) GRADUATE COURSES

CLB1404 CLB1405 CLB1401 CLB1603 LMP1407*

classroom hours

1995-96	3	4	3	10	
1996-97	3-	4	3	12	
1997-98	3	2	3	12	
1998-99					15
1999-2000					15
2000-2003					15
2004-2008					26h Annually

* Course co-ordinator, and co-lecturer, Introductory statistical analysis for postgraduate biomedical researchers. Sole instructor for course from 2004 onward.

Other Professional Activities,

Organizing Committee: 15th Workshop on Vitamin D – Houston Texas, June 19-20, 2012

Member of **Expert Advisory Panel for Canadian Academy of Health Sciences**, to advise Health Canada on the implementation of the 2011 Institutes of Medicine dietary guidelines for vitamin D and calcium.

Member of **Clinical Trial Steering Committee**. The SOLAR Trial of vitamin D in MS. Merck Serono. Golden Tulip Apollo Hotel, Amsterdam Apollolaan 2, 1077BA, Amsterdam, The Netherlands 6 March 2010, and January 2011.

Member **Data Safety and Monitoring Committee for Clinical Trial**: Establishment of the Vitamin D Requirements During Lactation. Medical University of South Carolina. Ongoing through 2009

Member Data Safety and Monitoring Committee for Clinical Trial: Evaluation of Vitamin D Status Through Maternal (Pregnancy) Supplementation. . Medical University of South Carolina. Ongoing through 2009

Consensus Group Member. American Geriatrics Society. Consensus Conference on Vitamin D for the Prevention of Falls and Their Consequences. Center for Disease Control, Atlanta GA. March 18-19 2009-2011

Organizing Committee: 14th Workshop on Vitamin D - Brugge, Belgium, 4 - 8 October 2009.

U of Toronto Faculty, Course coordinator, CME day on vitamin D. Faculty Club, Nov 2 2009

The Cholecalciferol Council, one of 7 experts for media consultation in the field of vitamin D http://www.cholecalciferol-council.com/vitamin_D_experts.htm

Osteoporosis Society of Canada, Scientific Advisory Panel Consultant, 2001- 2008.

***Member, Policy Development Group: EVIDENCE-BASED NUTRITION
WORKSHOP.*** Creighton University, Omaha NB. September 3, 2008

VIETH, UNIVERSITY TEACHING

Faculty of Pharmacy, University of Toronto,

Course Co-ordinator, Dr Reina Bendian,

Pathobiology Course PHM143, Lectures given annually since 1992.

Lecture 1. **Calcium, and Bone Disease**

Lecture 2. **Vitamin D: Fortification vs Supplement vs Prescription Drug**

VIETH BOOK CHAPTERS since 2015

The IOM—Endocrine Society Controversy on Recommended Vitamin D Targets: In Support of the Endocrine Society Position

Reinhold **Vieth**¹, Michael F. Holick² 1095

¹ University of Toronto, Toronto, ON, Canada; ² Boston University School of Medicine, Boston, MA, United States

In. Vitamin D, Volume 1: Biochemistry, Physiology and Diagnostics, Fourth Edition 2018. Chapter 57B pgs 1091-1107 <http://dx.doi.org/10.1016/B978-0-12-809965-0.00059-8>

Defining thresholds for vitamin D II

Reinhold **Vieth**, University of Toronto, Toronto, ON, Canada

In "Feldman and Pike's Vitamin D" 2023, *in press*

VIETH PUBMED-LISTED PUBLICATIONS 2015 TO 2023

UVB-emitting cloth to prevent low serum 25-hydroxyvitamin D caused by clothing.

Vieth R. Eur J Clin Nutr. 2023 Mar;77(3):405-406. doi: 10.1038/s41430-022-01253-9. Epub 2022 Dec 9. PMID: 36494476 No abstract available.

2

An Appraisal to Address Health Consequences of Vitamin D Deficiency With Food Fortification and Supplements: Time to Act!

Reichrath J, März W, DE Gruijl FR, **Vieth R**, Grant WB, Slominski AT, Holick MF, Vogt T, Pilz S. Anticancer Res. 2022 Oct;42(10):5009-5015. doi: 10.21873/anticancerres.16008. PMID: 36192012 Review.

3

Critique of Public Health Guidance for Vitamin D and Sun Exposure in the Context of Cancer and COVID-19.

Vieth R. Anticancer Res. 2022 Oct;42(10):5027-5034. doi: 10.21873/anticancerres.16011. PMID: 36191997 Review.

4

Mistakes in terminology cause false conclusions: Vitamin D does not increase the risk of dementia.

Vieth R. Aging Cell. 2022 Oct;21(10):e13722. doi: 10.1111/ace1.13722. Epub 2022 Sep 29. PMID: 36173739 Free PMC article.

5

Individual participant data (IPD)-level meta-analysis of randomised controlled trials with vitamin D-fortified foods to estimate Dietary Reference Values for vitamin D.

Cashman KD, Kiely ME, Andersen R, Grønborg IM, Madsen KH, Nissen J, Tetens I, Tripkovic L, Lanham-New SA, Toxqui L, Vaquero MP, Trautvetter U, Jahreis G, Mistry VV, Specker BL, Hower J, Knoll A, Wagner D, **Vieth R**, Öhlund I, Karlsland Åkeson P, Brett NR, Weiler HA, Ritz C. Eur J Nutr. 2021 Mar;60(2):939-959. doi: 10.1007/s00394-020-02298-x. Epub 2020 Jun 15. PMID: 32556447

6

Supplemental Vitamins and Minerals for Cardiovascular Disease Prevention and Treatment: JACC Focus Seminar.

Jenkins DJA, Spence JD, Giovannucci EL, Kim YI, Josse RG, **Vieth R**, Sahye-Pudaruth S, Paquette M, Patel D, Blanco Mejia S, Viguiliouk E, Nishi SK, Kavanagh M, Tsirakis T, Kendall CWC, Pichika SC, Sievenpiper JL. J Am Coll Cardiol. 2021 Feb 2;77(4):423-436. doi: 10.1016/j.jacc.2020.09.619. PMID: 33509399 Free article.

7

Vitamin D supplementation: cholecalciferol, calcifediol, and calcitriol.

Vieth R. Eur J Clin Nutr. 2020 Nov;74(11):1493-1497. doi: 10.1038/s41430-020-0697-1. Epub 2020 Jul 23. PMID: 32704098 Review.

8

Correction to: Weaker bones and white skin as adaptations to improve anthropological "fitness" for northern environments.

Vieth R. Osteoporos Int. 2020 Apr;31(4):803-804. doi: 10.1007/s00198-019-05251-9. PMID: 31970437 Free PMC article.

9

Weaker bones and white skin as adaptations to improve anthropological "fitness" for northern environments.

Vieth R. Osteoporos Int. 2020 Apr;31(4):617-624. doi: 10.1007/s00198-019-05167-4. Epub 2019 Nov 6. PMID: 31696275 Free PMC article. Review.

10

Neither human nor mouse is hypercalcaemic with 250 nmol/l 25-hydroxyvitamin D.

Vieth R. Brain. 2020 Feb 1;143(2):e9. doi: 10.1093/brain/awz388. PMID: 31848581 No abstract available.

11

The Paleolithic Nutrition Model in Relation to Ultraviolet Light and Vitamin D.

Vieth R. Adv Exp Med Biol. 2020;1268:409-419. doi: 10.1007/978-3-030-46227-7_21. PMID: 32918231 Review.

12

Randomized trial of daily high-dose vitamin D3 in patients with RRMS receiving subcutaneous interferon β -1a.

Hupperts R, Smolders J, **Vieth R**, Holmøy T, Marhardt K, Schluep M, Killestein J, Barkhof F, Beelke M, Grimaldi LME; SOLAR Study Group. Neurology. 2019 Nov 12;93(20):e1906-e1916. doi: 10.1212/WNL.0000000000008445. Epub 2019 Oct 8. PMID: 31594857 Free PMC article. Clinical Trial.

13

Randomized window of opportunity trial evaluating high-dose vitamin D in breast cancer patients.

Arnaout A, Robertson S, Pond GR, **Vieth R**, Jeong A, Hilton J, Ramsey T, Clemons M. Breast Cancer Res Treat. 2019 Nov;178(2):347-356. doi: 10.1007/s10549-019-05392-9. Epub 2019 Aug 9. PMID: 31399931 Clinical Trial.

14

Hypercalcemia and a "no observed adverse effect level" intake of vitamin D.

Vieth R. CMAJ. 2019 Jul 8;191(27):E768. doi: 10.1503/cmaj.72512. PMID: 31285382 Free PMC article. No abstract available.

15

25-Hydroxyvitamin D variability within-person due to diurnal rhythm and illness: a case report.

French CB, McDonnell SL, **Vieth R.** J Med Case Rep. 2019 Feb 4;13(1):29. doi: 10.1186/s13256-018-1948-9. PMID: 30712514 Free PMC article.

16

Rationale and Plan for Vitamin D Food Fortification: A Review and Guidance Paper.

Pilz S, März W, Cashman KD, Kiely ME, Whiting SJ, Holick MF, Grant WB, Pludowski P, Hilgsmann M, Trummer C, Schwetz V, Lerchbaum E, Pandis M, Tomaschitz A, Grubler MR, Gaksch M, Verheyen N, Hollis BW, Rejnmark L, Karras SN, Hahn A, Bischoff-Ferrari HA, Reichrath J, Jorde R, Elmadfa I, **Vieth R**, Scragg R, Calvo MS, van Schoor NM, Bouillon R, Lips P, Itkonen ST, Martineau AR, Lamberg-Allardt C, Zittermann A. Front Endocrinol (Lausanne). 2018 Jul 17;9:373. doi: 10.3389/fendo.2018.00373. eCollection 2018. PMID: 30065699 Free PMC article. Review.

17

Supplemental Vitamins and Minerals for CVD Prevention and Treatment.

Jenkins DJA, Spence JD, Giovannucci EL, Kim YI, Josse R, **Vieth R**, Blanco Mejia S, Viguiliouk E, Nishi S, Sahye-Pudaruth S, Paquette M, Patel D, Mitchell S, Kavanagh M, Tsirakis T, Bachiri L, Maran A, Umatheva N, McKay T, Trinidad G, Bernstein D, Chowdhury A, Correa-Betanzo J, Del Principe G, Hajizadeh A, Jayaraman R, Jenkins A, Jenkins W, Kalaichandran R, Kirupaharan G, Manisekaran P, Qutta T, Shahid R, Silver A, Villegas C, White J, Kendall CWC, Pichika SC, Sievenpiper JL. J Am Coll Cardiol. 2018 Jun 5;71(22):2570-2584. doi: 10.1016/j.jacc.2018.04.020. PMID: 29852980 Free article.

18

Sunbeds with UVB radiation can produce physiological levels of serum 25-Hydroxyvitamin D in healthy volunteers.

Kimball SM, Lee J, **Vieth R**. *Dermatoendocrinol*. 2017 Oct 17;9(1):e1375635. doi: 10.1080/19381980.2017.1375635. eCollection 2017. PMID: 29484099 Free PMC article.

19

A Randomized Double-Blind Placebo-Controlled Trial of the Effect of Vitamin D3 Supplementation on Breast Density in Premenopausal Women.

Brisson J, Bérubé S, Diorio C, Mâsse B, Lemieux J, Duchesne T, Delvin E, **Vieth R**, Yaffe MJ, Chiquette J. *Cancer Epidemiol Biomarkers Prev*. 2017 Aug;26(8):1233-1241. doi: 10.1158/1055-9965.EPI-17-0249. Epub 2017 May 17. PMID: 28515107 Clinical Trial.

20

Higher bone resorption excretion in South Asian women vs. White Caucasians and increased bone loss with higher seasonal cycling of vitamin D: Results from the D-FINES cohort study.

Darling AL, Hart KH, Gossiel F, Robertson F, Hunt J, Hill TR, Johnsen S, Berry JL, Eastell R, **Vieth R**, Lanham-New SA. *Bone*. 2017 May;98:47-53. doi: 10.1016/j.bone.2017.03.002. Epub 2017 Mar 7. PMID: 28286239 Free article.

21

microRNAs and DICER1 are regulated by 1,25-dihydroxyvitamin D in prostate stroma.

Dambal S, Giangreco AA, Acosta AM, Fairchild A, Richards Z, Deaton R, Wagner D, **Vieth R**, Gann PH, Kajdacsy-Balla A, Van der Kwast T, Nonn L. *J Steroid Biochem Mol Biol*. 2017 Mar;167:192-202. doi: 10.1016/j.jsbmb.2017.01.004. Epub 2017 Jan 12. PMID: 28089917 Free PMC article. Clinical Trial.

22

Effect of vitamin D supplementation on oral glucose tolerance in individuals with low vitamin D status and increased risk for developing type 2 diabetes (EVIDENCE): A double-blind, randomized, placebo-controlled clinical trial.

Moreira-Lucas TS, Duncan AM, Rabasa-Lhoret R, **Vieth R**, Gibbs AL, Badawi A, Wolever TM. *Diabetes Obes Metab*. 2017 Jan;19(1):133-141. doi: 10.1111/dom.12794. Epub 2016 Nov 9. PMID: 27717236 Clinical Trial.

23

Bioavailability and Safety of Vitamin D3 from Pizza Baked with Fortified Mozzarella Cheese: A Randomized Controlled Trial.

Al-Khalidi B, Chiu W, Rousseau D, **Vieth R**. *Can J Diet Pract Res*. 2015 Sep;76(3):109-16. doi: 10.3148/cjdpr-2015-015. PMID: 26280790 Clinical Trial.

Exhibit B

*FDA Color Report***Final Report of the Color Additive Scientific Review Panel¹**

Ronald W. Hart,² Stan C. Freni,³ David W. Gaylor,⁴ James R. Gillette,⁵ Larry K. Lowry,⁶ Jerrold M. Ward,⁷ Elizabeth K. Weisburger,^{8,12} Paul Lepore,^{9,11} and Angelo Turturro¹⁰⁻¹²

Received December 6, 1985; revised January 31, 1986

The Color Additives Scientific Review Panel considered whether there was information sufficient to perform a carcinogenic risk assessment on the colors D&C Red No. 19 (R-19), D&C Red No. 37 (R-37), D&C Orange No. 17 (O-17), D&C Red No. 9 (R-9), D&C Red No. 8 (R-8) and FD&C Red No. 3 (R-3) and to evaluate the assessments sent to FDA as part of the petitions for use of the colors for drug and external uses by the Cosmetic, Toiletry and Fragrance Association (CTFA). There is a lack of human data concerning the colors for making a human health assessment, so the assessments are based upon the extrapolation of animal data. The risk assessments are determined for exposure to single chemicals. Excluded from consideration are possible effects from exposure to multiple chemicals, such as co-carcinogenesis, promotion, synergism, antagonism, etc. In the light of recent efforts in establishing a consensus in risk assessment, the Panel has determined that the CTFA assessments for R-10, O-17, and R-9 are consistent with present acceptable usages, although it questions some of the assumptions used in the assessments. The Panel identified a number of general assumptions made, and discusses their validity, their impact on total uncertainty, and the potential options to address the gaps in understanding that necessitate the assumption. The Panel also derived revised risk estimates using more "reasonable" assumptions than "worst-case" situations, for 90th percentile and average exposure. For those assumptions that are easily quantifiable, the Panel's estimates are less than an order of magnitude lower than the CTFA risk estimates, indicating that the underestimates and overestimates of the CTFA risk estimates tend to balance each other. The impact of most of the assumptions is not quantifiable. The assessment for R-3 is complicated by the fact that there is no good skin

¹Because the original audience for this document was fairly broad it contained a good deal of background explanatory material and a number of summaries. For presentation here, the document has been shortened by removal of this material.

²Director, National Center for Toxicological Research, Jefferson, Arkansas 72079.

³DEHHE/Special Studies Branch, CDC/Center for Environ. Health, Chamblee 28-N, 1600 Clifton Road, Atlanta, GA 30333.

⁴Director, Biometry Staff, National Center for Toxicological Research, Jefferson, Arkansas 72039.

⁵Chief, Laboratory of Chemical Pharm., National Heart, Lung and Blood Inst., Bldg. 10, RM 8N117, 9000 Rockville Pike, Bethesda, MD 20205.

⁶Div. of Biomedical and Behavioral Science, National Inst. for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, OH 45226.

⁷Chief, Tumor Pathology and Pathogenesis Section, National Cancer Inst., FCRF, Bldg. 538/RM 205A, Frederick, MD 21701.

⁸Div. of Cancer Etiology, National Cancer Inst., Bldg. 31/RM 11A04, 9000 Rockville Pike, Bethesda, MD 20205.

⁹Office of Regulatory Affairs, FDA, 5600 Fisher Lane, Rockville, MD.

¹⁰Office of the Director, National Center for Toxicological Research, Jefferson, Arkansas 72079.

¹¹Executive Secretary.

¹²Editor of the text.

penetrance study for this color. It was assumed that the penetrance is similar to that of another water-soluble xanthene color, R-19. It is expected that the absorption of the color is not likely to exceed that of the smaller molecule, R-19. Therefore, the risk estimates are similar to the CTFA estimates, but with different reasoning. The estimates for R-8 and R-37 are different from the others in that there is a lack of any exposure or toxicological information on these colors. The toxicological properties were considered to be similar to the chemically related colors R-9 and R-19, respectively. The risk calculated for these dyes is actually a unit risk (i.e., a risk based on particular unit exposure). The unit exposure used is the exposure estimated from the chemically related color, which, from the production values for the dyes, is likely to have much higher usage. Exposure to all of these colors from external and drug uses is quite low, especially as a result of exposure through skin. Therefore, the risk estimates are low. It is cautioned that usage information is based on a recent survey, and changes in usage will alter the risk estimate.

KEY WORDS: Risk assessment; color additives; FD&C Red No. 3; D&C Red No. 8; D&C Red No. 9; D&C Red No. 19; D&C Red No. 37; D&C Orange No. 17; assumptions.

1. INTRODUCTION

The 1960 Color Additive Amendments to the Federal Food, Drug, and Cosmetic Act allowed temporary “listing” (i.e., permission to be marketed) of then-marketed color additives pending completion of studies to determine their safety.⁽¹⁾ The “provisionally listed” additives deemed safe were to be “permanently listed.” Approximately 200 color additives were involved, of which 126 have been “permanently listed” and 63 eliminated. Ten additives remain on the “provisional list”; six are considered in this report: D&C Red No. 19 (R-19), D&C Red No. 37 (R-37), D&C Orange No. 17 (O-17), D&C Red No. 9 (R-9), D&C Red No. 8 (R-8), and FD&C Red No. 3 (R-3).

An important part of the safety determination of these colors, because of the available toxicological evidence, is estimation of their carcinogenic risk. Information supporting continued marketing of the additives has been submitted to the Food and Drug Administration (FDA) by the Cosmetic, Toiletry and Fragrance Association (CTFA), that purports to show minimal risk from drug and cosmetic uses. These submissions include risk assessments for R-19,⁽²⁾ O-17,⁽³⁾ R-9,⁽⁴⁾ and R-3⁽⁵⁾ and an analysis of certain objections to these risk assessments.⁽⁶⁾ The CTFA analyses have been challenged on a number of bases, including scientific ones, with the suggestion that valid risk assessments cannot be performed on these colors.⁽⁷⁾

In order to insure the use of the best science in FDA determinations, and as part of a continuing

process of peer review, the Commissioner of Food and Drugs charged a Panel consisting of knowledgeable scientists from a number of Federal agencies to evaluate: (1) the possibility of performing a scientifically valid carcinogenic risk assessment of the six colors for drug and cosmetic uses; and (2) whether the available information supports the risk analyses before the FDA. These deliberations were done against the background of recent reports which are developing a consensus on risk assessment.⁽⁸⁻¹⁰⁾

2. CTFA RISK ASSESSMENTS

2.1. Summary of CTFA Risk Assessments

In very brief summary, the submitted CTFA risk assessments contain:

1. Chemical identification of the six colors and their impurities.
2. *Hazard Identification* —Description of *in vitro*, short-term, and long-term animal assays including two-generation studies, used for both evidence for carcinogenicity and in deriving risk estimates. This was done for four colors (R-3, R-9, R-19, O-17), and assumed that the response of R-37 was similar to R-19, and R-8 similar to R-9 on an equal color basis.
3. *Exposure Assessment* —Including: a listing of color-containing generic cosmetic products with color concentration; estimates of the amount of product per application; assess-

ment of usage using two surveys; estimation of the color applied available for skin absorption; assessment of human skin penetrance using an *in vitro* model.

4. *Dose-Response Assessment* —Using positive cancer bioassays and a linearized multistage model.
5. *Risk Characterization* —Discussion of important assumptions such as:
 - a. Animal cancer response can be extrapolated to humans using a metameter of mg/kg-day;
 - b. Dose-response can be extrapolated from high doses used in the animal experiments to the low doses seen in humans;
 - c. The colors absorbed through the skin would evoke the same response, qualitatively and quantitatively, as colors absorbed on an equal absorbed dose basis;
 - d. The total absorbed dose per day equals the frequency of application times the concentration of color in the products times the amount of product per application times the color resorption rate.

It was also suggested that the uncertainties in the risk assessments were approached using conservative assumptions (i.e., assumptions that err on the safe side and were unlikely to underestimate risk).

2.2. Evaluation of CTFA Risk Assessments

The Panel basically agrees with the risk assessment methodology used by CTFA, but it questions some of the assumptions used in the analyses. Many assumptions are explicitly stated, others become apparent only after a careful consideration of CTFA's line of reasoning, or of the methodology used. A number of these assumptions are commonly made in conducting a risk assessment. Use of an assumption by CTFA does not necessarily imply that it considered the assumption valid. Indeed, many of the assumptions were made for the explicit purpose of deriving what the CTFA characterized as a "conservative risk estimate" by overestimating exposure, and were often followed by a discussion reflecting CTFA's awareness that the assumption was erroneous. The assumptions in the CTFA assessments are listed below, with the Panel's analysis, followed

by revised estimates for the risk in each color-specific section.

Since, in certain cases, generating data rather than making assumptions may provide information which can reduce the number of necessary assumptions and their associated uncertainties, suitable and feasible studies are sometimes suggested.

3. GENERAL ASSUMPTIONS USED IN THE RISK ASSESSMENTS

3.1. General Assumptions Used in the Hazard Identifications

- *Assumption 1:* Any carcinogenic response in an animal test predicts a carcinogenic response in humans.

Validity: Unknown. It has been shown that all human carcinogens are also animal carcinogens in some assay. However, relatively few animal carcinogens have been shown to be carcinogenic in humans. This may reflect either lack of, or deficiencies in, design and implementation of epidemiologic studies. On the other hand, humans may be insensitive, or the animal model may not mirror the human response. For its deliberations, the Panel did not evaluate the weight of evidence for human carcinogenicity since it understood that it was FDA policy to consider an agent which is carcinogenic in some experimental paradigm, in any species, as a suspect human carcinogen. If the weight of evidence would have been evaluated, there is the possibility that one or more of the colors would not have fulfilled some accepted criteria (e.g., IARC criteria⁽¹¹⁾) to be considered human carcinogens. In this framework, the Panel accepted the use of the above assumption.

- *Assumption 2:* The carcinogenic potential of a chemical is independent of route of administration and the medium used (i.e., if the colors are shown to be carcinogenic after oral exposure, it is assumed that skin application would induce cancer as well unless there is evidence to the contrary).

Validity: Testable by conducting chronic assays by skin application. Skin carcinogenesis tests have been uniformly negative for the colors of concern. However, as the amount of material expected to penetrate the skin in these tests was low compared to the amounts tested in the ingestion assays, the Panel accepted use of the assumption, attempting to adjust

for differential exposure resulting from skin penetrance compared to oral exposure (see also assumption 2 in section 3.2 below).

- **Assumption 3:** An animal bioassay is more appropriate for hazard identification if the chemical is tested at or near the maximum tolerated dose (MTD). It is assumed that critical metabolic pathways are not overwhelmed and carcinogenic mechanisms are the same regardless of dose.

Validity: Unknown and, at the current state of the science, difficult to test. Generally, animals are placed on test when 6–8 weeks of age for a chronic bioassay. To increase assay sensitivity, multigeneration protocols are also employed; in such studies the animal is exposed at all stages of development, including organogenesis and rapid proliferation, presumably more sensitive stages of development. The use of this protocol requires an expanded definition of the MTD since a toxic dose and toxic effect for an embryo may be very different from that for an adult organism. Although this was not done, the Panel used this assumption.

3.2. General Assumptions Used in the Exposure Assessments

3.2.1. Nonproduct-Specific General Assumptions

Humans are exposed to the colors of concern by means of cosmetics, drugs for skin application, and products for personal hygiene. The Panel chose to derive two different estimates of exposure: (1) the 90th percentile exposure values (i.e., equal to a value that is more than or equal to that which is expected for 90% of the population affected, understanding that it was a long-standing FDA practice to use this value); and (2) an approximate “reasonable” estimate, based on the anticipated average or mean exposure.

- **Assumption 1:** Orally administered or ingested colors are absorbed equally well in test animals and man, regardless of the test concentration of the color and the vehicle used.

Validity: Unknown; it is testable, although rarely done in risk assessments.

Option: Conduct comparative absorption studies in test animals and man.

Impact: The quantitative impact of the assumption on the risk estimate is unknown, it may result in under- or overestimation of the risk. The Panel accepted use of the assumption.

- **Assumption 2:** In comparing routes of administration, the amount of color which penetrates the skin will be equally effective as the amount which penetrates the gastrointestinal tract in evoking a carcinogenic response.

Validity: Unknown and testable. There is good evidence that gastrointestinal microflora and skin can activate carcinogens by very different mechanisms (see also assumption 2 in section 3.1).

Option: Conduct studies which deliver similar systemic doses by various routes in appropriate animal model systems.

Impact: Depends upon carcinogenic mechanism and agent metabolism, which have not been characterized well. The Panel accepted use of the assumption.

- **Assumption 3:** Relative absorption in humans can be estimated under standardized experimental conditions using 2–5 days postmortem abdominal skin specimens.

Validity: Unlikely to be valid, testable. The assumption is based upon the implied claim that a postmortem skin specimen does not differ from live skin with regard to permeability, that skin preparation can be standardized, and that abdominal skin represents other areas of the body as well.

The skin penetration studies^(12–15) use an *in vitro* skin test model consisting of a 1- or 2.5-cm² section of human skin mounted in a diffusion cell. The dermal surface of the skin was placed in isotonic saline at pH 7.4 and 37°C. The bathing solution (the receptor phase) was removed and sampled at regular intervals. For some colors, it was replaced by a Volpo-20 solution to increase color solubility. The colors were applied in various concentrations and vehicles (simulating, to some degree, the composition of commercial products), in volumes of 5, 10, or 25 μ l, or as 25 mg talc. The radioactively labeled colors were left *in situ* for periods of 2–3 days, and the absorption of color through the skin into the receptor phase was measured with a scintillation spectrometer. The skin sections were obtained from the abdominal skin of deceased hospital patients within 24 h of their death, and used either immediately or refrigerated for no more than 20 h. Before mounting in the diffusion cells, the subcutaneous fat tissue and approximately 50% of the dermis was removed by shaving with a scalpel. In a number of cases, the epidermis was also tested (separately from the dermis), in order to investigate the effects of differential retention on tissue permeability.

A number of factors are important in estimating human percutaneous absorption, including the application of *in vitro* to *in vivo* results, site of application, and individual variation.⁽¹⁶⁾ The percent dose absorbed of triclocarbon (sic, triclocarban) was significantly different in a static *in vitro* system compared to an *in vitro* continuous flow system.^(16,17) Since the paradigm involved changing the bathing solution and altering vehicle to simulate the solubilizing effects of blood flow, the experimental system used was probably more similar to the continuous flow system than a static diffusion cell. Since the continuous flow system appears to be a fairly good model for *in vivo* human skin for triclocarban, and the *in vitro* models overestimated the *in vivo* penetrance of another chemical (paraquat), the Panel determined that although there appears to be significant variation with different chemicals the *in vitro* model was not likely to significantly underestimate skin penetrance.

Options: Conduct rigidly standardized *in vivo* and *in vitro* studies using live skin at various sites (in particular facial and scalp).

Impact: There are insufficient data available to assess the quantitative impact of using postmortem skin sections, but the model is very likely to overestimate the true absorption rate. Since postmortem skin disintegrates with time, it is likely that the skin's integrity as a barrier will be affected. The longer the time since death, the more permeable the skin becomes. Another aspect of the skin model is that artificial canals between the skin surface and the receptor phase have been created by cutting through the sweat and hair ducts. The effect of this artifact has not yet been quantified but will result in raising the observed absorption rate. On the question of differential absorption as a function of site of application, for a few chemicals tested such as parathion or malathion, the urinary excretion of labeled material as percent of applied dose from jaw or forehead is less than three times higher than in glabrous abdominal skin.⁽¹⁸⁾ Scrotum and vulval skin absorb more than the forehead.⁽¹⁶⁾ Other experiments looking at the absorption of hair dyes under practical conditions of application indicate that an important factor is the surface area of the hair in the region, and it is suggested that the 75–100 times larger surface area of hair on the scalp is the main factor in the relatively low scalp penetration of hair dyes (0.02%) that penetrate 100 times more in the ventral aspect of the forearm.⁽¹⁹⁾ From these data, it is likely that penetra-

tion rates for the colors under consideration vary in different areas of skin. In order to model absorption through all skin areas, especially for products used all over the body (e.g., bath products, soaps, etc.), the Panel used the data from the tests conducted on abdominal skin and multiplied by an overall average absorption value of three. This is probably a conservative estimate.

● **Assumption 4:** For each color, the skin absorption rate equals the steady-state rate, unless this is not reached after 3 days, in which case twice the maximum rate at that time is used to approximate the absorption rate.

Validity: Seems reasonable for the steady state, but not for the maximum rate and is testable. Much depends on how well the *in vitro* skin test reflects reality. It is hard to understand a continuous rising trend in the absorption rate through healthy and live skin, but it is easy to understand for a skin section 5 or more days postmortem. Since it may be expected that with increasing time postmortem, a degenerating skin will become more penetrable to the colors, the absence of a steady state may suggest the presence of an artifact.

Option: Repeat the skin tests with live skin for extended periods of time.

Impact: Since the absence of a steady state after 3 days may reflect a postmortem skin artifact, the assumption that twice the maximum rate represents the true absorption rate probably results in overestimating the exposure. The Panel considered this assumption, when relevant, in the color-specific sections.

● **Assumption 5:** The CTFA-sponsored usage frequency surveys are representative of the usage pattern in the population of users of cosmetics and drugs for external use.

Validity: Probably incorrect and testable. CTFA conducted two surveys on the use of cosmetics and hygiene products. The usage surveys included a one-week prospective survey of 47 female CTFA employees and their families, and a retrospective survey among 1129 customers of a retail chain run by a major cosmetics producer. Data were available to the Panel for R-3, R-9, R-19, and O-17.^(2–5) The prospective study included CTFA female personnel and their families, while the retrospective study involved clients of a cosmetics retail chain only. It is not clear whether male clients were also included. It is very likely, in both surveys, that males have been underrepresented, while there is an overrepresentation of

frequent users. Further, interviewees of the retrospective survey are likely to be limited to products of one manufacturer only and, thus, have less variation in products (and, consequently, in dose levels) to choose from.

Options: Conduct surveys among the general population and provide data by age category (e.g., infants, older children, adults, elderly) and gender.

Impact: In general, the prospective study appears to be less biased than the retrospective study. It also yielded an approximately 15% lower usage frequency than did the retrospective study. It is unknown to what extent exposure is overestimated by the nonrepresentativeness of both surveys with regard to the population of product users. In the absence of more appropriate data, the Panel accepted use of the CTFA assumption, appreciating that it overestimates the risk. Since the prospective study seems to be less biased, when possible, exposure was determined with data from this survey.

- **Assumption 6:** For each color, the exposure is for a lifetime.

Validity: Obviously invalid and testable.

Options: Either: (a) make a more appropriate estimate based on practical considerations, and consider males separate from females; or (b) provide realistic estimates for each product based on surveys among the general population (as opposed to the clientele of these products); or (c) provide a realistic estimate for all products combined, based on surveys among the general population.

Impact: If exposure started, on the average at age 10, exposure would be for 6/7 of an average lifetime. The Panel used 6/7 of the CTFA exposure estimate, also accepting the assumption that the relationship between exposure and risk is independent of the age at exposure.

- **Assumption 7:** People use only products that contain the carcinogenic colors of concern.

Validity: Obviously invalid and testable.

Option: Conduct a survey on actual use of colored and uncolored products in the general population. It is appreciated, however, that patterns of use can change with time.

Impact: The available data are not sufficient for an accurate impact assessment. In order to reflect the wide variety of products available and the production figures of these colors compared to all the colors, the Panel used an arbitrary factor of one half to "correct" the assumption. However, more accurate data will probably revise this figure downward. This 0.5 factor is not likely to underestimate the risk and

is assumed to be more reasonable than assuming 100% use of products containing carcinogenic colors.

- **Assumption 8:** People use only products with the highest concentration of the color.

Validity: Invalid and testable.

Option: Conduct a market survey on the concentration of products.

Impact: The survey of products containing R-19 cited in the risk assessment⁽²⁾ indicates that the average concentration used in a product is less than 1/4 of the maximum concentration permitted in commercial products. The Panel used this factor for R-19 and, given the lack of any other information, also assumed that this is true for the other colors of concern.

- **Assumption 9:** The daily absorbed amount of color per product used equals:

$$a \times b \times c$$

where a = the amount of color in the product per application; b = the frequency of application (following the usage survey); and c = the percent absorbed per day of applied amount.

Validity: Unknown, it can be challenged.

Option: Conduct *in vivo* and *in vitro* (with live skin) studies and measure the amount available for absorption (rather than the amount applied), the amount applied per cm², the surface covered by the product, for varying frequencies of application per day with soluble and insoluble colors.

Impact: Since the value of c depends on many variables, such as the equilibrium between penetration rate and tissue retention, the amount of color lost by wiping or wearing off (i.e., the difference between the amount applied and the amount available for absorption), the thickness of the applied layer of product, the diffusion rate of color in this layer, etc., there may be either an under- or over-estimation of exposure, by using the above approximation. Typically, cosmetics are regularly removed by cleaning or wearing off. The amount applied to skin in the diffusion cell did not account for the loss of material occurring *in vivo* (e.g., by wiping off excessively applied cosmetics, wearing off or rubbing off, and cleaning). Estimates of this loss of material can be made but are necessarily crude in the absence of adequate test data. If the cosmetic is not immediately reapplied, the absorption will probably drop because the steady-state absorption rate, reached *in vitro* after three days, will probably not be reached (this consideration is especially germane to

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the more insoluble colors which reach the steady-state absorption rate much more slowly than the others). Thus, the use of the steady-state rate in the above equation would overestimate exposure. However, a model which is based on one application in 2–3 days, when the real-life situation includes more frequent applications, will underestimate exposure because frequent application will cause the average concentration of the thin color layer directly in contact with the skin to have a higher average concentration (it doesn't become as depleted and as dependent on diffusion from the more color-rich layers above to replenish it in a short time as opposed to 2–3 days). This is, again, especially significant for the insoluble colors since the diffusion rate (and replenishment) will be slow. Since no information is available to support and/or quantify the above considerations, the Panel accepted use of the CTFA assumption.

- **Assumption 10:** For each color the total exposure is the sum of exposures to all products containing the color of concern.

Validity: A reasonable and testable assumption.

Option: Test mixtures rather than single colors.

Impact: Although unlikely, it is possible that there is interaction between the colors or that there is some change in skin penetration as a function of the number of colors being present. Interaction with the various kinds of vehicles may lead to either under- or overestimation. However, the overall impact is not likely to be substantial. The Panel used the assumption.

- **Assumption 11:** The amount of consumer product per application is 5–10 mg/cm².

Validity: Unknown, but testable assumption. In the CTFA risk assessments, values have been listed without reference and lacking information about distribution.

Option: Establish a database on the amounts of color used per application and the skin area exposed, through a general survey of the population.

Impact: Since there is no information, this assumption cannot be assessed. It may over- or underestimate exposure. The Panel accepted use of the CTFA assumption.

- **Assumption 12:** Differences in the vehicles used in the skin absorption studies are not likely to result in changes in the absorption of more than 10–30%.

Validity: Unknown but testable. There may be little similarity between the vehicle used in the absorption studies and the actual test product [e.g., nail polish uses vehicles which ensure fast evaporation to form a tough water- (and serum-) insoluble film].

Option: Expand the skin studies to include vehicles more similar to those used in consumer products.

Impact: Without data, no accurate estimate can be made of the effect of the test vehicle. There are no data to support the estimate of a 10–30% variation, nor is there any information on the direction of the difference. In the absence of data, the Panel used the CTFA assumption.

- **Assumption 13:** In an appropriate vehicle, there is no difference between a color in a formulation used as a primary color or in a lake form (frequently used in cosmetics, a lake is the salt of the color extended on a substratum of alumina, clay, titanium dioxide, etc.).

Validity: Unknown, but testable. Making a lake presumably makes the color more insoluble, thereby decreasing the unbound concentration which governs the rate of absorption. This prevents color leaching, is useful in cosmetic applications and may change skin penetrance.

Option: Conduct skin absorption studies using both lakes and primary colors.

Impact: There are no data available for assessing the impact. Since the lakes are made to restrict the water solubility of the color, their use would probably result in less absorption through the skin. In the absence of data, the Panel accepted use of the assumption.

- **Assumption 14:** That the major constituent of the color is the one of primary toxicological concern and can be modeled by the skin absorption test.

Validity: Reasonable and testable.

Option: Repeat skin absorption studies and evaluate the impurities for differential absorption and carcinogenic “potency.”

Impact: No data available for an accurate impact assessment. The colors are not single chemical entities despite their categorization with simple chemical names (e.g., R-19 is frequently termed Rhodamine B). FDA certification requirements are generally not rigorous. They do not fully characterize the purity of the color (as percent of color additive) or the amounts and variety of impurities that may be present. For example, methods to determine “% color,” often confused with “% purity,” are based on the reduction of the color with titanium trichloride or the determination of total color, by weight, using spectrophotometry. Thus, subsidiary colors (colored analogs which are closely related chemically) would usually be included with the “pure color” as “% color” by these nonspecific methods. The certifica-

tion requirements also specify the levels of some starting materials, volatiles, organic solvent-extractable compounds, subsidiary colors, salts, and inorganic contaminants. The methods used in certification are often neither state-of-the-art nor chemically definitive. In addition to impurities allowed by the certification process, FDA,^(20–26) Environ,⁽⁶⁾ and others^(27–32) have speculated on the large variety of *possible* impurities that may be present as a result of side reactions during synthesis, products from impure starting materials, and in some cases color-derived degradation products.

The Panel judged that the major issue concerning impurities was not those that were possible, but those that were actually quantitated in FDA certified lots, particularly in those lots used in the carcinogenicity bioassays. The impact of these impurities on the risk assessments depends not only on their concentrations, but also on their carcinogenic “potency” and, for external uses, skin penetrance relative to the major color additive. Reasonably definitive analyses have been reported for these certified lots by more rigorous methodology than that required by the certification requirements. In terms of impurities actually present, the contaminants of major concern are the subsidiary colors and the residual starting materials.

For the subsidiary colors, even if they were carcinogenic and present at 5% by weight (the maximum reported amount), close similarity in chemical structure to the main color would probably preclude large differences in skin penetration rates and/or potency. Thus, the risk assessment remains basically unaltered.

For the residual starting materials, the highest concentrations found have not exceeded 1% by weight, with those chemicals related to known mutagens or carcinogens (such as the aromatic amines) not exceeding 0.5% by weight. In the special case where the color additive may be metabolized to a split product (such as an aromatic amine, and this material is present as a starting material impurity), the bioavailable aromatic amine in humans would be the sum of the dermally absorbed impurity and the metabolic product of the dermally absorbed color additive. Estimating the potency and penetrance of the actual impurities, the Environ Report⁽⁶⁾ and the other available data indicate that it is highly unlikely that these impurities could be totally responsible for the reported carcinogenic effect or that the contribution from the starting material would be significant

compared to that available from the metabolism of the intact color additive.

More quantitatively, the residual starting materials are unlikely to be more than five times as “potent” (i.e., equivalent in effect to five times the amount of major component), and 10 times as capable of penetrating the skin, as the major color component. If this “worst case” is so, their maximal effect can be estimated, in terms of exposure, as follows:

It is useful to define a μg toxic-color-equivalent ($\mu\text{-tce}$) as the color-equivalent (in μg) $\times$ “potency” $\times$ the absorbed %. In these terms, if the color is pure major component (and subsidiary colors) (with “potency” 1) for a gut absorption of 50%, one mg color equals 500 $\mu\text{-tce}$. In the skin, with absorption of 1%, 1 mg equals 10 $\mu\text{-tce}$. Assuming pure color, the “dose” from skin exposure of 1 mg is therefore expected to be 10/500 of the same amount in the ingestion studies. However, maximally, 1 mg of commercial color used in the ingestion studies consists of 995 μg of major (and subsidiary color) components of “potency” 1, and, 5 μg (0.05%) of starting material with “potency” 5. This equals 497.5 $\mu\text{-tce}$ (from the major component) + 12.5 $\mu\text{-tce}$ (from the starting materials), or 510 $\mu\text{-tce}$. Similarly, in skin, 1 mg of maximally contaminated color, assuming 1% penetrance of the major component (and subsidiary colors) and, again maximally, skin penetrance of 10% for the more “potent” contaminants results in 9.95 $\mu\text{-tce}$ (from the major components) and $5 \times 5 \times 0.10 = 2.5$ $\mu\text{-tce}$ (from the starting residuals) or a total of 12.4 $\mu\text{-tce}$. Therefore, the “dose” expected as a result of skin exposure from 1 mg of color with maximal impurity contamination could be equivalent to as much as 12.4/510 of that amount in the ingestion studies [i.e., a factor of $(12.4/510 \div 10/500)$ or 1.22 times the exposure].

Thus, to account for significant impurities (starting materials and subsidiary colors), a factor of 1.22 times the CTFA exposure estimate was used.

3.2.2. Product-Specific Assumptions

- *Assumption 1:* For hair cosmetics, 2% of the applied color reaches the skin (i.e., the ratio of skin area to hair area is assumed to be 1:50).

Validity: The total surface of hair is 75–100 times the surface of the scalp. Using 1:50 seems conservative if the total hair surface is indeed 75–100

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times the skin surface, a figure which may be an underestimation for females.

Option: Compile data on hair length, diameter, and density.

Impact: No data available for an accurate estimation. If the estimate is too low, the risk estimate is proportionally too high. Since the skin/hair surface ratio of 1:75, or 1.33% reaching the skin, may already be high, further raising it to 1:50 appears to be an unnecessary application of a safety factor ranging from $2/1.33 = 1.5$ to (for a ratio of 1:100) $2/1 = 2.0$. The Panel used what it considered to be a more appropriate figure, 1.75%, or approximately 1:60.

- *Assumption 2:* For nail products, 1% of the applied color reaches the skin or the cuticle.

Validity: Reasonable and testable, but it still does not account for vehicle volatility.

Option: Conduct a test of nail products under realistic conditions.

Impact: Since the solvents in nail polish are highly volatile and nail polish is intended to dry up to an insoluble film almost immediately after application, the penetration rate of colors in such products is virtually zero. Therefore, it is very unlikely under this condition that a measurable color absorption would occur in the very brief period of application. Theoretically, absorption of color from nail creams and lotions is possible. But the test model did not evaluate nails as the area of application. Given the low frequency use of this product⁽²⁻⁵⁾ and, in view of the above discussion, assuming a zero absorption of colors from nail products is, in practice, not an underestimation of the risk. The Panel assumed zero absorption from nail products.

- *Assumption 3:* For bathwater products, 2% of the applied color reaches the skin. It is assumed that a layer of 1 mm of water, equaling 1.8 l of water, would cover the body to form the source from which color can be absorbed; this is 1.8% of an assumed 100 liters of bathwater.

Validity: Reasonable and probably testable.

Option: Conduct an *in vivo* study of animals immersed in a color solution.

Impact: Unknown due to lack of data, but not likely to be significant. The Panel accepted the assumption used by CTFA.

- *Assumption 4:* Products used for a short period only (wash-off products) have an absorption of 50% of that of products in continuous use, regardless of how short the actual exposure was.

Validity: Seems incorrect and is testable.

Option: Repeat skin test focused on amount of color retained in the skin during various short periods of time.

Impact: The skin test data⁽¹²⁻¹⁵⁾ suggest that the absorption for wash-off products is far less than 50% of that for products with continuous use. Hence, exposure is considerably overestimated. Since an absorption rate of 25% of the rate for products used continuously seems more realistic, the Panel used a correction factor of 0.5 for the CTFA exposure estimation. More information is required for a more accurate quantification of the assumption.

- *Assumption 5:* For products other than wash-off products it is assumed that the amount applied equals the amount available for absorption.

Validity: Incorrect and testable. It is likely that in most applications of cosmetics they are applied in excess and they wear or are rubbed off. Therefore, the amount left for absorption is less than the amount applied. The overestimate of exposure is related to the amount removed (i.e., not available for absorption).

Option: Conduct studies focused on measuring the proportion of applied product that is either removed by wiping off or by contact with materials other than skin (e.g., clothes).

Impact: The amount of color available for absorption is hard to quantitate. It seems reasonable, however, to assume 50% loss for products other than wash-off products; therefore, the Panel used a factor of 1/2. This correction factor should not be applied to products in a water-alcohol vehicle.

3.3. General Assumptions Used in the Dose-Response Assessments

- *Assumption 1:* In long-term rodent-feeding studies, there is 100% absorption of the colors.

Validity: Unknown and is testable. The colored feces seen in some studies suggest that some color was not absorbed. However, because of processes such as biliary excretion, elimination of the compound in the feces does not necessarily indicate lack of absorption. Some of the benzidine azo dyes, which also resulted in colored feces, demonstrated low absorption when given by the oral route. A likely absorption range is 25–75%.

Option: Conduct absorption studies for oral administration.

Impact: Unknown, and may lead to an underestimate of risk for externally used products. If only 50% of the carcinogen is absorbed from the feed, then tumors are produced by 1/2 of the administered dose. Hence, the CTFA risks for colors absorbed through the skin should be multiplied by 2, a factor the Panel used. No correction is needed for internal exposure from swallowed lip products, if the proportion absorbed by the oral route is similar in humans and rodents.

- **Assumption 2:** If a color is carcinogenic in humans, the risk of cancer for a lifetime exposure in humans is equal to the risk for a lifetime (approximately two years) exposure in rodents for equal daily doses on a body weight basis (mg/kg body weight-day).

Validity: Probably not true. Hoel *et al.*⁽³³⁾ suggested the use of mg/m² body surface area-day on the basis of the acute toxicity of anticancer drugs in humans.⁽³⁴⁾ Crump *et al.*,⁽³⁵⁾ using a data base derived from the NTP bioassays for over 200 chemicals, concluded that the dosage measurement giving the closest correlation between species was mg/kg body weight-day. However, use of relations such as these has led to concern. It has been noted that

It does not appear safe to generalize concerning rates of unknown metabolic reactions. Many exceptions could be found to the apparent tendency of intrinsic rate to decrease with increasing body size: Quite different qualitative pathways may dominate in different species and toxicity may sometimes correlate with the concentration of an active intermediate that represents only a minor elimination pathway.⁽³⁶⁾

It should be appreciated that studies on the distribution and metabolism of the principal components of the colors have been performed only for R-3 and R-9. Even these studies are not sufficiently extensive to decrease markedly the uncertainties in making the risk assessment. To achieve accurate estimates of risk, one requires knowledge of the metabolites and the mechanisms by which a carcinogen causes an increase in malignant tumors. Many interrelated factors will govern the incidence of carcinogenesis in a given study. Among these are species, strain, and individual differences in the relative distribution of enzymes that govern the formation of adducts in different tissues. Dose-dependent kinetics may occur, but the effects at different doses will be unpredictable. Environmental factors will also affect the fraction of the dose of the carcinogen that is converted to relevant metabolites. Oncology has not progressed to the understanding of all these

factors even for the animals used in bioassays.⁽³⁷⁾ Even if these parameters were known for the bioassay, however, the probable influence of different environmental and genetic factors, and the heterogeneity in the distribution of these factors in the human population, should be appreciated.

There is no direct evidence to assess the relative carcinogenic potency of these colors between humans and rodents. However, for 15 carcinogenic agents, after defining "potency," relative "potencies" between humans and the most sensitive rodent data were generally within a factor of 20, either high or low, when dose was measured in terms of mg/kg body weight.⁽³⁸⁾ To generalize, for an individual chemical, the risk estimate calculated could be low or high by a factor of 100. Quantitatively, extrapolation on a surface area basis would multiply the CTFA risk estimates by approximately 7 for those based on rats and 12 for mice.

Option: Would require epidemiological data. Since anticipated cancer risks are small, an extremely large study would be required. Exposure would be difficult to measure and is not practical. Alternatively, pharmacokinetic analyses in combination with knowledge of the carcinogenic metabolites may be adequate to define a good metameter.

Impact: Unknown, could be substantial in either direction. The Panel used this assumption.

- **Assumption 3:** For extrapolation, a woman is considered to have a lifetime average body weight of 53 kg.

Validity: Probably valid for the general female population, unknown for the population of cosmetic users.

Option: Conduct a survey of weights among the product users.

Impact: A value of 60 kg as a lifetime average weight of the general population (including males) is commonly used by the FDA. Since the products are likely to be used predominantly by females, with males unlikely to be exposed to products having significant color concentrations (aftershave lotion, bath soap, etc., have very low color concentrations), it seems reasonable to accept 53 kg for the risk calculation. The Panel accepted use of the CTFA assumption.

- **Assumption 4:** The linearized multistage model provides an upper bound on low dose risk estimates in the convex portion of the dose-response curve.

Validity: Probably provides an upper bound on low dose risk estimates for the rodent species.⁽³⁹⁾ The appropriate model to choose for "best" estimates is

unknown. Various models may fit the experimental data equally well, but may differ considerably at the lower doses. Even if the multistage model were appropriate, whether or not a linear term is included makes a considerable difference at the lower doses. If a linear term is present, the “best estimate” may be within a factor of two of the upper-bound estimate. If a linear term is not present, however, the “best” estimate may be several orders of magnitude below the upper-bound estimate. Thus, even restricting estimation to the multistage model cannot establish a “best” estimate because the high dose experimental data cannot establish the presence or absence of low-dose linearity. Therefore, “best” estimates should not be attempted as was done by CTFA even for the rodent species. The use of a model incorporating low-dose linearity is consistent with a recent suggestion by the OSTP carcinogen document.⁽⁹⁾

Option: Other linearized models could be used, but they are not likely to vary much from the linearized multistage model estimates.

Impact: If these colors act to add to carcinogenic processes already existing in humans, then low-dose linearity is expected. The choice of the model then offers no additional safety assurance and does not impact on the risk estimates. If the colors act by independent processes to produce cancer and low-dose linearity does not result, the upper-bound estimates may be higher than actual risk by several orders of magnitude. The Panel used this assumption.

● *Assumption 5:* The most sensitive animal tumor data (species-strain-sex-tissue or organ site) are the most appropriate indicator for human sensitivity and are generally used to estimate risk.

Validity: No direct evidence that this would or would not be a reasonable representation of very sensitive human subpopulations.

Impact: Unknown, could be substantial. The best animal model to predict cancer incidence in the “most sensitive” human subpopulations is seldom known and would probably vary with the carcinogen being considered. The Panel accepted use of this assumption, in accord with a recent suggestion.⁽⁹⁾

3.4. Risk Characterization

When the assumptions used in the CTFA risk assessments have been accepted by the Panel, it is frequently because there seem to be no alternatives with higher degree of validity for the uncertainties involved, and because some are consistent with what

the Panel understands is FDA policy. The risk estimates given by the Panel are based on reasonable estimates of exposure, whenever the Panel believed that it was possible to make such an estimate. In situations in which available data would allow for a choice between “degrees of reasonable estimate,” the Panel consistently selected the estimate associated with the higher risk, following prudent public-health policy.

For example, the use of an upper 95% confidence limit for the linearized multistage model utilized in the low-dose extrapolation probably leads to an overestimate of risk for rodents. If we also assume equal susceptibility for rodents and humans, and using the most tumor-sensitive site and species in conjunction with conservative estimates of exposure, the estimates presented are likely to lead to an overestimate of risks for humans.

3.5. Conclusion on the Total Impact of Assumptions on the Risk Estimate

In the following sections, the product- and color-specific assumptions are applied to the usage data contained in the CTFA risk assessments to derive the Panel exposure values (e.g., if absorption through the use of nail polish for a color was assumed to contribute 0.0002 µg/day to the total exposure, the use of the correction factor of zero results in this amount becoming zero). The Panel used these assumptions with survey estimates of 90th percentile exposure (the Risk/90 values) and average and “reasonable” estimates of exposure (Risk/Rea) to derive the revised risk estimates.

For the general assumptions above which are approximately quantifiable, for skin exposure, the correction factor accounts for:

- i. General Exposure Assumption 3 (section 3.2.1.): 3
- ii. General Exposure Assumption 6 (section 3.2.1.): 6/7
- iii. General Exposure Assumption 7 (section 3.2.1.): 0.5
- iv. General Exposure Assumption 8 (section 3.2.1.): 0.25
- v. General Exposure Assumption 14 (section 3.2.1.): 1.22
- vi. General Dose-Response Assumption 1 (section 3.3.): 2

$$(3 \times 6/7 \times 0.5 \times 0.25 \times 1.22 \times 2) \\ = 0.8 \times \text{CTFA estimate}$$

For incidental ingestion of lip products, parameters relevant only to skin exposure or not relevant to lipstick products drop out (all except *ii*):

$$(6/7) = 6/7 \times \text{CTFA estimate}$$

At low dose levels, the estimated cancer risk, as computed with the linearized multistage risk model, is directly proportional to the dose levels.⁽⁹⁾ Therefore, appropriate risk estimates, based on the adjusted exposure estimates (i.e., modified by the product and color-specific assumptions) are multiplied by the above correction factors to derive the Panel risk estimates.

The Panel recognizes that the correction factor of 0.8 for skin absorption is inconsequential compared to the uncertainties in the assumptions that are difficult to quantify. Less than half of the 24 identified assumptions are even approximately quantifiable, some only with fairly arbitrary factors. The correction factor for skin absorption does *not* mean that the risk estimate is precise within 20% of the actual human risk. What it does mean is that, for the various quantifiable assumptions, underestimates and overestimates of risk in the CTFA analyses essentially cancel out.

The assumptions, and the risk assessments that derive from them, are directed at estimating the effects from exposure to a single chemical only. They do not include possible effects of exposure to multiple chemicals such as co-carcinogenesis, promotion, synergism, or antagonism. Finally, when absorbed dose is cited to three or more significant figures, the use of this many figures does not imply precision; it is merely a mathematical tool to avoid cumulative rounding errors.

4. D&C RED NO. 19 (R-19)

4.1. Chemistry

D&C Red No. 19 (CI 45170) (R-19) (CAS 81-88-9) is the water-soluble xanthene dye 9-(2-carboxyphenyl)-3,6-bis(diethylamino)xanthylium chloride (FDA also terms it the 3-ethochloride of 9-o-carboxyphenyl-6-diethylamino-3-ethylimido-3-isoxanthene). Its common name is Rhodamine B and its structure is shown in Fig. 1.^(20,40)

R-19 is synthesized from the condensation of phthalic anhydride and 3-diethylaminophenol to produce a colorless lactone intermediate, which is saponified and acidified to produce the hydrochloride pigment.⁽²⁰⁾ The synthetic pathway is shown in Fig. 2.

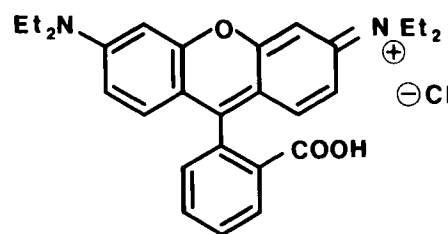


Fig. 1. Structure of major component of R-19.

FDA certification requires that each color lot meet the following specifications⁽⁴¹⁾:

- Volatile matter, not more than 5.0 percent.
- Water-insoluble matter, not more than 1.0 percent.
- Ether extracts (from acid solution), not more than 0.5 percent.
- Dimethyl-m-aminophenol, not more than 0.2 percent.
- Chlorides and sulfates of sodium, not more than 1.0 percent.
- Pure dye (as determined by titration with titanium trichloride), not less than 92.0 percent.

According to an FDA report,⁽²⁰⁾ the impurities likely to be present include phthalic anhydride, phthalic acid, diethyl-m-aminophenol, resorcinol, o-(2-hydroxy-4-diethylaminobenzoyl)benzoic acid, and triethylrhodamine. Side reaction and degradation products were listed as possibly present but in low concentrations. The Environ Report⁽⁶⁾ states that possible impurities include the starting materials, phthalic anhydride and diethyl-m-aminophenol, and the intermediate o-(2-hydroxy-4-diethylaminobenzoyl)benzoic acid, as well as the subsidiary color, triethylrhodamine. In addition, simple aromatic amines that could be extracted in ether are conceivable. Commercial lots of color contain triethylrhodamine and o-(2-hydroxy-4-diethylaminobenzoyl)benzoic acid. An HPLC method with reverse phase chromatography and detection at 230 nm showed the absence of starting materials in commercial colors, but the presence of peaks described as subsidiary colors.⁽²⁷⁾ Some of the impurities are of toxicological significance since it has been shown that impurities after separation by Thin Layer Chromatography (TLC) are mutagenic by the Ames test.⁽⁴²⁾

If the FDA specifications are met and the methods for the determination are sufficiently rigorous for screening purposes, then the levels of these organic impurities should not exceed 2–4% by weight. The likelihood that the total level of impurities is confined to a single compound is low. Most of the impurities seem to be composed of the subsidiary color, triethylrhodamine.⁽²⁰⁾

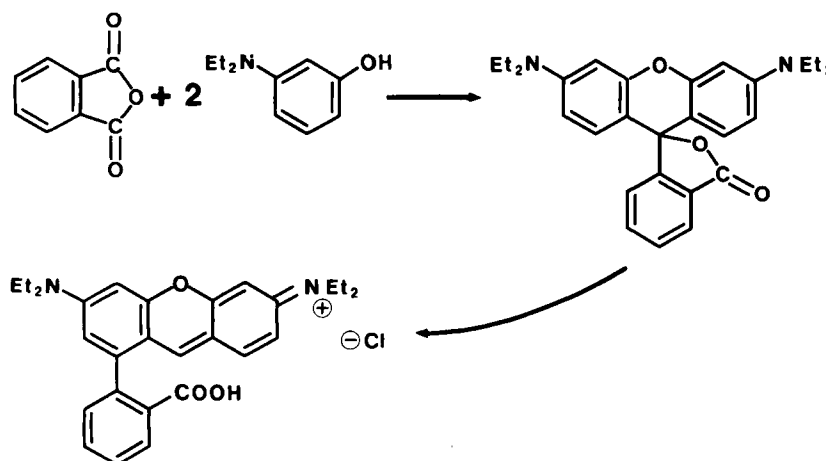


Fig. 2. Synthesis of major component of R-19.

4.2. Short-Term Tests

4.2.1. Mutagenicity

R-19 gave no response in the bacterial Ames tests, using tester strain of *Salmonella typhimurium* TA98 in a sample from FDA,⁽⁴³⁾ in TA98 or TA1535 using a sample from a major textile firm,⁽⁴⁴⁾ or in a sample from Merck Chemical Co. over a wide dose range in TA98 or TA100⁽⁴⁵⁾ (a report that the color was inactive in a number of other strains was considered problematical because the color was inhibitory to the bacteria under the conditions used in the assay⁽⁴⁶⁾). Color supplied by the Sigma Chemical Co.⁽⁴²⁾ or in a rhodamine dye tablet (used to trace water flow),⁽⁴⁷⁾ however, was mutagenic in TA98, TA100, and TA1537, as was a lake in TA98, TA1537, and TA1538.⁽⁴⁸⁾ The key to understanding these data was provided by the observations that when another color source, with less impurities, was used or the impurity separated by fractionation,⁽⁴²⁾ the color was not mutagenic, indicating that the positive response seemed to be a result of impurities. In higher organisms, the color did not induce gene conversion in yeast,⁽⁴⁹⁾ although there was a weak mutagenic effect when the color (without fractionation) was added to food for *Drosophila*.⁽⁵⁰⁾ From these studies it appears that the mutagenic activity of this color is contained in impurities.

4.2.2. DNA Damage/Repair

A *rec* test in *E. coli* was negative,⁽⁵¹⁾ while positive induction of DNA breaks in the *in vitro*

Chinese Hamster Ovary cells (CHO) assay was suggested to be related to the lethality of Rhodamine B at the doses used or impurities.⁽⁴²⁾ The color did not induce gene conversion in yeast,⁽⁴⁹⁾ an index of recombinative activity. In CHO cells, DNA damage does occur,⁽⁵²⁾ but at doses with significant cell lethality.⁽⁵³⁾ The lethality is not surprising since the color has been shown to be phototoxic, at lower doses, to cultured human skin cells.⁽⁵⁴⁾ The CHO studies were conducted using “gold” lights⁽⁵⁵⁾ which remove the ultraviolet and blue portion of the spectrum. However, the photoactivation spectrum for the toxic effect of the color is well into the visible region,⁽⁵⁴⁾ which would not be removed by “gold” fluorescent illumination. It appears, therefore, that color phototoxicity is an important factor in its ability to damage DNA.

4.2.3. Chromosomal Damage

Chromosomal aberrations were not found in a human cell line at R-19 concentrations up to 10^{-4} M,⁽⁵⁵⁾ or in onion root tip or broad bean in culture.⁽⁵⁶⁾ However, clastogenic effects were found in Muntjac chromosomes,⁽⁵⁷⁾ and CHO cells.^(52,58) No induction of bone marrow sister chromatid exchanges (SCE) in the mouse was found at high doses.⁽⁵⁹⁾

For the study with Muntjac cells, the color was obtained from Sigma Chemical Co., a source which was shown to contain mutagenic impurities.⁽⁴²⁾ It was not purified before use. In one of the positive CHO studies,⁽⁵²⁾ the color was fractionated before use. However for a significant effect, the dose is

approximately at the concentration at which half the cells die (the LC_{50}),⁽⁵³⁾ under conditions of “gold” illumination (discussed above). In the other positive CHO study, cellular toxicity of the color was not reported.⁽⁵⁸⁾ However, if it is similar to that seen in the study by Douglas *et al.*,⁽⁵²⁾ it was appreciable at the effective doses. Therefore, although the color has clastogenic effects, they could be a result either of an impurity in the color or of color toxicity.

4.2.4. Transformation

No reported assays.

4.2.5. Significance

The results suggest that the observed genetic toxicity of R-19 may be a result of noncolored impurities in commercial preparations or may be an indication of R-19 toxicity, especially phototoxicity. Because of this, Rhodamine B should be handled properly when assayed for genetic toxicity.

From the information available in the literature, it is not clear what are the stability characteristics of fractionated color. Fractionated color appears to be nonmutagenic and only damages DNA in doses high enough to induce cell lethality.

4.3. Comparative Toxicology

4.3.1. Negative Studies

Rat: R-19 was fed to Wistar rats (5 male and 5 female) at 4% of the diet for 2 years. No toxic or oncogenic effect was noted.⁽⁶⁰⁾

R-19 was fed to 21 male and female Wistar rats at 0.13% of the diet for 7 months and then at 0.2% in a lifetime study. No significant effects were noted.⁽⁶¹⁾

R-19 was also fed at 0.008, 0.025, and 0.15% of the diet to groups of 25 male and 25 female SD rats for 104 weeks. Growth suppression was noted in male and female rats at 0.025 and 0.15% levels. Male rats at the 0.008% level also had lower body weights from about the 40th week. There were increases in the thyroid weights and thyroid/body weight ratios.⁽⁶²⁾

R-19 was fed to 25 male and 25 female Charles River rats at 0.004% of the diet for 26 months and no adverse effects other than pink coloration of the eyes were noted.⁽⁶³⁾

R-19 was fed to Long-Evans rats at 0.04, 0.4, and 4 mg/kg in the diet in a three-generation study, each generation involving 10 males and 20 females at each dose level. No oncogenic effect was noted.⁽⁶⁴⁾

R-19 was fed to CD rats (60 males and 60 females/group in F_0 and 70 males and 70 females at F_1) in diets containing 0.002, 0.005, and 0.02%. In this two-generation study that continued for 30 months, body weights were somewhat lower in high-dose females.⁽⁶⁵⁾

Dog: R-19 was fed to 3 male and 3 female beagle dogs per group at 0.008, 0.0325, and 0.1% of the diet for 2 years. No oncogenic effect was noted.⁽⁶⁶⁾

Skin Painting: In mice, 0.1 ml of a 1% solution of R-19 was painted twice weekly on the backs of 50 male and 50 female ICR Swiss-Webster mice for 18 months. No oncogenic effect was noted.⁽⁶⁷⁾

4.3.2. Positive Studies

Rats: In CD rats, a multigenerational study similar in protocol and numbers to the one that found no significant effect at 0.02% used 0.075% of R-19 in which the animals were dosed for 132 weeks with *in utero* exposure.⁽⁶⁸⁾ Animals receiving 0.075% had a significant reduction in body weight and a significant increase in follicular cell tumors of the thyroid in treated males, both adenomas (12/56 compared to 2/57 for controls) and carcinomas (5/56 compared to 1/57).

Mouse: CD-1 mice (60 animals/sex/group) were dosed at levels of 0.005, 0.02, and 0.1% in the diet for 24 months. Hepatocellular carcinomas were increased in females, up to 19/60 for 0.1% R-19 for adenomas and carcinomas (compared to 4/115 for controls).⁽⁶⁹⁾

4.3.3. Interpretation

Interpretation of the positive rat study:

Toxicology. Rats fed R-19 at 0.075% had moderate weight depression but no effect on survival was seen. Females had more severe weight depression. Toxic lesions in the potential target sites, thyroid and parathyroid glands, were not seen.

Pathology. Increased incidence of thyroid follicular tumors (adenomas and carcinomas) was found in 0.075% males (17/56) as compared with controls (3/57). Five of the dosed males had carcinomas while one control male had thyroid follicular

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carcinoma. Goiter was not reported. In females, 6/55 dosed rats had thyroid follicular tumors and 2/57 were found in control females. Parathyroid tumors were reported in 9/48 dosed males and 1/44 control males. Few chemicals, if any, cause these parathyroid tumors. Since it is easy to misdiagnose parathyroid hyperplasia (due to renal disease) as neoplasia, these lesions should be reviewed histologically.

Conclusion. This study showed R-19 led to tumors in male rats. More severe body weight depression was noted in females which did not have induced tumors. Goiter was not noted.

The R-19 was obtained from Sun Chemical Corporation as a FDA certified lot, with a stated "purity" of 92%, lot AA-4989. No data were included on the further characterization of the purity of the color additive or the presence of impurities. A later FDA report indicated that lot AA-4989 was 90.3% color, 2.8% volatiles, 0.08% insolubles, and 2.3% triethylrhodamine.⁽²⁰⁾ A supplementary submitted report⁽²⁸⁾ showed that lot AA-4989 of the color contained 1.58% triethylrhodamine, expressed as the ratio of triethylrhodamine to R-19 at 554 nm, and about 0.4% other colored impurities, expressed as the ratio of impurities to R-19 at 554 nm. This report did not mention noncolored impurities. From the point of view of chemical purity of the dosing material, the studies were performed in an acceptable manner except for the lack of sufficient data on the purity of the color and the impurities present.

Interpretation of the positive mouse study:

Toxicology. The feeding of diets containing R-19 at a level of 0.1% did not produce effects on body weight gain or mortality. Mild toxic liver lesions were found in mice.

Pathology. Increased incidences of liver tumors were found in both sexes. Dosed males (0.1%) had an incidence of 15/59 as compared with 17/119 in the control males, while 0.1% dosed females had an incidence of 19/60 vs. 4/115 in the control females. More than 50% of the liver tumors in dosed mice of both sexes were malignant.

Conclusion. This study showed that feeding R-19 increased the incidence of liver tumors in female mice. Since no effect was found on body weight gain or survival of both sexes, it is possible that the mice could have tolerated higher doses of R-19. The mild toxic liver lesions in male mice would also support this position.

The chemical analysis of the dosing material used in, and the conclusions of the analysis about,

the mouse study were identical to the positive rat study since they were done together.

4.4. Skin Penetration Studies

The *in vitro* studies in human abdominal skin⁽¹²⁾ used ¹⁴C-labeled R-19 obtained from Amersham Corporation as "side rings-U-(¹⁴C)" Rhodamine B, having a specific activity of 38 mCi/mmol (79 μ Ci/mg). The presumed location of label is shown in Fig. 3.

TLC assays for radiochemical purity, reported by the supplier, using two solvent systems, indicated 95 and 96% purity. Additional TLC assays were also performed.⁽²⁸⁾ Three TLC bands were separated, scraped, eluted, and counted. The results showed 92.6% Rhodamine B, 5.48% triethylrhodamine, and 1.96% "other" determined from the radioactivity found in eluted bands. When quantitation of the bands was based on absorption at 554 nm, the results showed 97.1% of Rhodamine B, 2.0% triethylrhodamine, and 0.9% "other." Noncolored impurities would not be detected by the latter method. Specific activities calculated on a total weight basis were 85.3 and 81.7 μ Ci/mg as compared to 79 μ Ci/mg reported by the supplier.

Radioactive color was prepared in aqueous formulations of different pH, as well as in alcohol, oils, emulsions, and talc. Doses varied from 0.001% in aqueous formulations to 0.5% in talc. Appropriate amounts of ¹⁴C were applied to approximately 1 cm² of human abdominal skin mounted in the Franz cell with receptor saline solution.

Recoveries from aqueous buffers and emulsions were good with significant retention in the epidermis. Recoveries from oils were also good with significant amounts removed with the first wash. No recoveries were performed with talc.

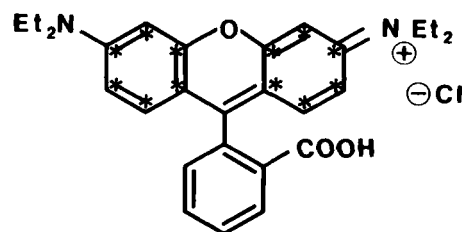


Fig. 3. Presumed location of radiolabel in R-19 major component.

Results showed a 2% pH-related penetration of color in aqueous buffers with smaller penetration in emulsions, oils, and talc. The radiochemical purity was well-defined in the supplementary report.⁽²⁸⁾ There are still questions as to impurities present and the nature of the ^{14}C component that was found in the receptor cells, but these questions are largely academic and would have a relatively small impact on risk assessment when using the assumptions discussed above.

4.5. Specific Assumptions used in the CTFA R-19 Risk Assessment

- *Assumption 1:* Emulsions are assumed to be absorbed at a rate of 1%/day of the amount applied, which is the rate seen for aqueous solutions. The observed rate of 0.24% may be too low since it was unclear whether (a) the observed rate was the steady state rate; and (b) emulsions in commerce contain the soluble color or the lake.

Validity: Probably invalid, it is easily testable.

Option: Conduct new studies on emulsions for an extended period of time, and include live skin specimens.

Impact: The skin penetrance study⁽¹²⁾ shows that the steady state was reached after approximately 60 h. The argument that emulsions contain the color rather than the lake is irrelevant, since the test was performed with the color. Moreover, since it is already questionable whether 3–5 days postmortem skin would retain its original integrity, the assumption of a rate of 1%/day implies a safety factor of 4. The Panel assumed that the absorption rate of colors in emulsions equals 0.25% per day of the amount applied to the skin.

- *Assumption 2:* Since a steady state was not reached with mineral oil as a vehicle, an absorption rate of twice the maximum rate after 3 days is assumed for oil-based products (0.5% for mineral oil and castor oil).

Validity: Unlikely to be correct, it is testable.

Options: (a) Conduct *in vivo* tests in humans and suitable animals; (b) repeat *in vitro* test for extended periods of time with live skin.

Impact: Using twice the absorption rate of mineral oil (0.5%/day) for products based on castor oil is incorrect since the latter rapidly reached a steady-state rate of 0.04%. The assumption implies the application of a safety factor of 12.5. If post-

mortem artifacts prevent mineral-oil-based colors from reaching the steady state, risk estimates based on the test results are considerably too high. The Panel assumed a reasonable estimate of the mineral-oil absorption rate, given the results in postmortem skin, equal to the observed maximum rate of 0.25% (rounded) and, for castor oil products, no reasons have been found to depart from the observed steady-state rate of 0.04%.

- *Assumption 3:* The absorption rate for aqueous products is assumed to be 1%/day of the applied amount, except for those products that need adjustment.

Validity: Seems correct based on the *in vitro* data.

Impact: The penetrance studies show a range of steady-state absorption rates of 0.82 to 1.03%/day, where 1.03% refers to a 0.001% aqueous solution at pH = 8. Since 0.001% is the maximum concentration of the commercial products and not a median or average, this assumption is one that will probably overestimate the rate. Without other data, however, the Panel accepted use of the CTFA assumption.

- *Assumption 4:* The absorption rate for talc products is 0.002%/day of the amount applied.

Validity: Seems correct based on the *in vitro* data.

Impact: The above rate is a steady-state rate achieved as soon as 2 h after application. Thus, the effect of further deterioration of the skin with time is minimal. The Panel used the CTFA assumption.

4.6. Risk Characterization

4.6.1. CTFA Risk Estimates

From the BIO/DYNAMICS Study in CD-1 Mice. The risk was slightly higher using liver tumors (adenoma or carcinoma) rather than hepatocellular carcinoma only. The estimates of the upper confidence limit on low-dose risk from the linearized model (but not the tumor incidences) were comparable in males and females and, therefore, the tumor data were combined.

From the BIO/DYNAMICS studies in Charles River CD rats. The risk was somewhat higher using thyroid follicular cell tumors (adenoma or carcinoma) rather than carcinoma only. Risk estimates for males and females were similar and, therefore, the tumor data combined.

Table I. Comparison of Risk Estimates of R-19

	Risk(CTFA/90) ^a	Risk(CTFA/Avg) ^b	Risk/90 ^c	Risk/Rea ^d
Rat	2.7×10^{-7}	7.1×10^{-8}	1.1×10^{-7}	2.8×10^{-8}
Mouse	1.3×10^{-7}	3.4×10^{-8}	0.5×10^{-7}	1.3×10^{-8}

^aRisk(CTFA/90) is the CTFA risk estimate at the upper 90th percentile of exposure.

^bRisk(CTFA/Avg) is the CTFA risk estimate at "average" estimates of exposure.

^cRisk/90 is the risk estimate based on the Panel's calculation at the 90th percentile of exposure.

^dRisk(Rea) is the risk estimate based on the Panel's calculation of a more reasonable estimate of exposure.

4.6.2. Panel Risk Estimates

Application of the Panel product- and color-specific assumptions above to the usage data⁽²⁾ results in a total absorbed amount of color of: 0.42 $\mu\text{g}/\text{day}$ as an average of the prospective usage survey; 1.62 $\mu\text{g}/\text{day}$ as the upper 90th percentile of the prospective survey, as compared to CTFA's estimates of 0.85 $\mu\text{g}/\text{day}$ and 3.27 $\mu\text{g}/\text{day}$, respectively. (The factor 0.8 to account for the general assumptions was used.)

4.6.3. Comparison

See Table I.

4.7. Summary

Studies have been conducted on TLC-separated colors that showed some impurities were mutagenic by the Ames test, while the purified color fraction had little indication of mutagenicity. The levels of these impurities may have an impact on R-19 carcinogenicity. However, because of their relatively low concentration, their presence is not likely to alter the risk assessment calculations of risk of the entire color by a significant degree.

Although the risk of R-19 is low, the impurities may be responsible for some of its toxicity. Therefore, it is recommended that the impurities be removed before it is used. It should be possible to develop simple techniques which are cost-effective, and inclusion of a purification step as a criteria for certification, removing those impurities which have significant mutagenic action, would probably reduce an already small risk.

Finally, the color has been shown to be photo-toxic. Conditions of its use and the possibility of

effects as a result of light exposure should be considered.

5. D&C RED NO. 37 (R-37)

5.1. Chemistry

D&C Red No. 37 (CAS 6373-07-5) (CI 45170:1) (R-37) is an alcohol soluble xanthene color 9-(2-carboxyphenyl)-3,6-bis(diethylamino)-xanthylium octadecanoate (FDA also terms it the 3-ethostearate of 9-o-carboxyphenyl-6-diethylamino-3-ethylimido-3-isoxanthene). Its common name is Rhodamine B stearate and its structure is shown in Fig. 4.^(20,70) It is the stearate salt of the same cation as R-19.

R-37 is synthesized from R-19 by treatment with ammonium hydroxide and stearic acid.⁽⁷⁰⁾

FDA certification requires that each color lot meet the following specifications⁽⁴¹⁾:

- Volatile matter, not more than 2.0 percent.
- Water-insoluble matter, not more than 1.0 percent.
- Sulfated ash, not more than 3.0 percent.
- Matter insoluble in benzene, not more than 0.5 percent.
- Dimethyl-m-aminophenol, not more than 0.2 percent.
- Stearic acid (not part of the dye), not more than 50.0 percent.
- Pure dye (as determined by titration with titanium trichloride), not less than 50.0 percent.

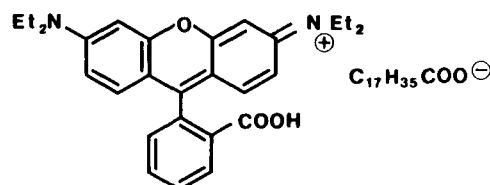


Fig. 4. Structure of major component of R-37.

Therefore, approximately half the molecular weight is taken up by noncolored stearate anion.

FDA⁽²⁰⁾ describes impurities likely to be present as phthalic anhydride, phthalic acid, diethyl-m-aminophenol, stearic acid, fatty acids related to stearic acid, o-(2-hydroxy-4-diethylaminobenzoyl)benzoic acid, and triethylrhodamine. A variety of possible side reaction and degradation products were listed as possibly present, but in low concentrations. The Environ Report⁽⁶⁾ does not address R-37, but any impurities are expected to be similar to those reported for R-19. Commercial lots were shown to contain triethylrhodamine, stearic acid, and o-(2-hydroxy-4-diethylaminobenzoyl)benzoic acid. Using an HPLC method, an absence of starting materials, and the presence of 6–7 peaks considered to be subsidiary colors has been reported.⁽²⁷⁾ Some of the impurities are likely to be of toxicological significance since those separated from the closely related R-19 by TLC are mutagenic.⁽⁴²⁾

If the FDA specifications are met and the methods for the determination are sufficiently rigorous for screening purposes, then the levels of these organic impurities, less stearic acid when totaled, should not exceed 2–4% by weight. The likelihood that this total level of impurities is confined to a single starting material is low, with most of the impurities seemingly confined to the subsidiary color, triethylrhodamine.⁽²⁰⁾

5.2. Short-Term Tests

5.2.1. Mutagenicity

In addition to the information on R-19, R-37 was found not be mutagenic, with or without activation, in TA98, TA100, TA1535, and TA1537.⁽⁴⁶⁾

5.2.2. Other

No additional information.

5.2.3. Significance

R-37 is negative in a mutagenic assay in a laboratory that also found R-19 to be negative. There is no reason to believe that the color will be less contaminated with impurities than the parent R-19, except that the presence of the stearic acid will dilute the contaminant.

5.3. Comparative Toxicology

No long-term animal assays have been performed with this color additive. The presence of the stearate is not likely to affect the toxicological effects of the color once absorbed, except by simple dilution of the cation concentration. It is not clear whether the moiety will decrease gastrointestinal absorption because of decreased water solubility, or increase it as a result of ion-pairing effects.

5.4. Skin Penetration Studies

No skin penetration studies have been performed with this color additive. It is not clear whether the moiety will decrease skin penetrance because of decreased water solubility, or increase it as a result of ion-pairing effects.

5.5. Specific Assumptions Used in the R-37 Risk Assessment

- *Assumption 1:* The information on R-19 can be used to predict the risk from R-37.

Validity: Reasonable and testable. Although it can be assumed that once absorbed, the stearate salt would dissociate to R-19, the issue of concern is the absorption. R-37 might be expected to be more slowly absorbed because of its larger molecular weight and its decreased water solubility. In addition, because of the stearate moiety, certified lots of R-37 only contain about 50% cation by weight compared to more than 92% color for R-19. However, there is a question that ion-pairing effects might alter the absorption rate. Therefore, it is prudent to assume, in the absence of specific information, that the response will be similar to that seen for R-19.

Option: Conduct tests on the toxicity and skin penetrance of R-37.

Impact: The direction and degree of absorption and risk change is not clear. The dilution by the stearic acid will reduce risk by a factor of 2 for the same weight of material. However, if twice as much material (the same amount of “color”) is used in applications, this factor will disappear. There is no information on usage of R-37.

5.6. Risk Estimates

5.6.1. CTFA Risk Estimates

CTFA does not estimate risk from this color.

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5.6.2. Panel Unit Risk Estimates

Since no color-specific information appears to be available with regard to usage frequency, *in vitro* skin absorption, and commercial formulation, and because R-37 differs from R-19 only in the added stearate moiety, R-19 data on toxicology and skin absorption have been accepted as likely to represent R-37, using a factor of 0.5 for dilution of cation by the R-37 stearate moiety.

It must be appreciated that there is no exposure information (and little toxicological information) for this color, and the risk estimate is based on simple extrapolation from a chemically related color. Thus, the nature of this risk estimate is different from those calculated for R-19, O-17, R-9, and R-3. A risk estimate of 1.3×10^{-7} is actually a unit risk for an absorbed exposure of 0.062 $\mu\text{g/day}$ to R-19-type colors. There is no information on actual human exposure, other than the production of the color is much less than the production of R-19.

5.7. Summary

In the few systems tested, there is no evidence that this color is genetically toxic. It is reasonable to assume that R-37 has the same toxicological effects as R-19, except that the unit risk is divided by approximately two to provide for an equal weight of cation. The unit risk estimates should be used to calculate absolute risks as soon as usage data become available, with absorption studies of color-specific absorption to adjust the risk.

6. D&C ORANGE NO. 17 (O-17)

6.1. Chemistry

D&C Orange No. 17 (CAS 3468-63-1) (CI 12075) (O-17) is a water-insoluble monoazo pigment 1-[(2,4-dinitrophenyl)azo]-2-naphthalenol (also termed 1-(2,4-dinitrophenylazo)-2-naphthalenol by the FDA).

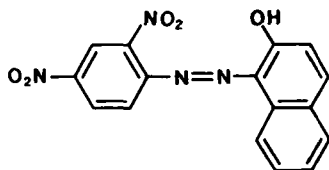


Fig. 5. Structure of major component of O-17.

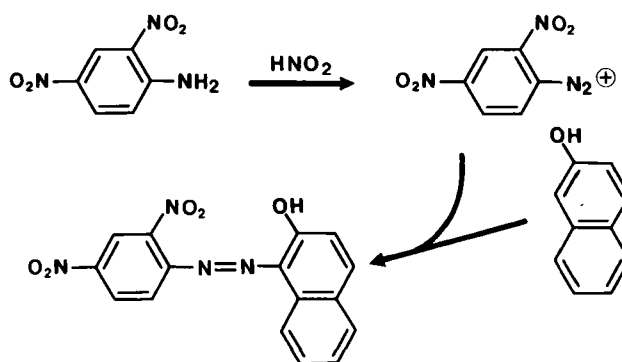


Fig. 6. Synthesis of major component of O-17.

A common trade name is Permanent Orange and its structure is shown in Fig. 5.⁽⁷¹⁾

O-17 is synthesized by the condensation of the diazonium salt of 2,4-dinitrobenzeneamine (2,4-dinitroaniline) with 2-naphthalenol (2-naphthol, beta-naphthol). The synthetic pathway is shown in Fig. 6.⁽²¹⁾

FDA certification requires that each color lot meet the following specifications⁽⁴¹⁾:

- Volatile matter, not more than 5.0 percent.
- Sulfated ash, not more than 1.0 percent.
- Matter insoluble in toluene, not more than 1.5 percent.
- 2,4-dinitroaniline, not more than 0.2 percent.
- Beta-naphthol, not more than 0.2 percent.
- Pure dye (as determined by titration with titanium trichloride), not less than 90.0 percent.

FDA⁽²¹⁾ describes the impurities likely to be present as 2-naphthalenol (< 0.2%), 2,4-dinitrobenzeneamine (< 0.05%), 4-[(2,4-dinitrophenyl)azo]-1-naphthalenol (< 0.05%), 2-[(2,4-dinitrophenyl)azo]-1-naphthalenol (< 0.005%), 1-[(4-nitrophenyl)azo]-2-naphthalenol (< 0.05%), and possibly a trace of 1-[(2,6-dinitrophenyl)azo]-2-naphthalenol. Other side and degradation products were listed as possibly present, but at trace concentrations. The Environ Report⁽⁶⁾ states that the most likely impurities are the starting materials 2,4-dinitrobenzeneamine and 2-naphthalenol, although small quantities of ether-extractable nitroaromatics are conceivable. An HPLC method for the determination of the two starting materials showed much higher levels of 2,4-dinitrobenzeneamine and 2-naphthalenol⁽²²⁾ than by the official FDA cellulose column chromatography method, primarily because the HPLC method solubilized the “insoluble” color and released more impurities trapped in the crystal structure. These results imply a higher level of starting materials in

FDA certified lots than reported by the official method.

Commercial pigment lots were shown to contain the two starting materials and the first three subsidiary colors listed above. The starting material 2,4-dinitrobenzeneamine, a nitroaromatic amine, has been shown to be mutagenic in the Ames test, with and without rat liver S-9 activation.⁽⁷²⁾

6.2. Short-Term Tests

6.2.1. Mutagenicity

This color is mutagenic in TA98, TA100, TA1535, A1537, and TA1538 with or without S9 activation,⁽⁴⁹⁾ with increased activity with microsomal activation except for TA1535. It is also mutagenic whether the source of color is FDA,⁽⁴³⁾ a major textile firm,⁽⁴⁴⁾ in rouge and lipstick,^(43,46) or from other sources.⁽⁷³⁾ Microsomal activation has been shown to increase⁽⁴⁹⁾ or inhibit^(43-45,73) the response, the difference in reaction to the microsomal mix perhaps reflecting impurities.

In the forward mutation assay using the TK locus in L5178Y mouse lymphoma cells, O-17, from 0.65 to 5 mg/ml, is inactive⁽⁷⁴⁾ with and without S-9 activation.

6.2.2. Other

There were no data for DNA damage/repair, chromosomal damage or transformation.

6.2.3. Significance

This color is a mutagen in bacteria, although not in the one mammalian system tested. There may also be contaminants in the preparation which may be responsible for the difference in the effects of microsomal activation. However, the reduction product 2,4-dinitrobenzeneamine is mutagenic and doesn't require microsomal activation,⁽⁷²⁾ possibly because the bacteria possess nitroreductases. Azo reduction to a nitrobenzeneamine may be the source of much of the activity. Whatever the source, it is clearly an agent which damages the genome.

6.3. Comparative Toxicology

6.3.1. Negative or Equivocal Studies

Mouse: CD-1 mice (60 animals/sex/group) were dosed with 0.025, 0.25, and 1.0% O-17 in the diet for 23–25 months. There was an increase in hepatocellular neoplasms in males, but the increase was not significant.⁽⁷⁵⁾

Rat: O-17 was given at 0.025, 0.1, and 1% of the diet to groups of 25 male and 25 female CD Charles River rats for 104 weeks. Some growth suppression was noted at the higher level. Liver weights were increased in higher dose females. Neoplastic nodules of the liver and hepatocellular carcinomas were increased in females at the 1% dose level, though not significantly.⁽⁶³⁾

Dog: O-17 was fed to 3 male and 3 female beagle dogs per group at 0.025, 0.125%, or 1% of the diet for 2 years. In general the treated dogs showed lower body weight gains than controls, however, no significant pathological effects were noted.⁽⁶⁶⁾

Skin Painting Studies: In mice, 0.1 ml of a 1% solution of O-17 was painted on the skin of 50 male and 50 female ICR Swiss-Webster mice twice weekly for 18 months. No oncogenic effect was noted.⁽⁶⁷⁾

0.1 ml of a 1% solution of O-17 was applied weekly to the skin of 50 male and 50 female CF-1 Carworth mice for up to 26 months. No oncogenic effect was noted.⁽⁷⁶⁾

6.3.2. Positive Study

Rat: O-17 was given at 1% of the diet initially to F₀ (60 per sex/group) and then the F₁ generation (70/sex/group) of Charles River rats for 26–30 months. This was an FDA-requested study by the same contractor using the same methodology of doses of 0.02, 0.05, and 0.1% of O-17 in diet. Mortality of treated rats was increased over that of controls in the second year. Male rats had an 18% depression in body weight. Pericholangitis was greater in treated male rats killed at 12 months. A similar effect was noted in female rats but at terminal sacrifice the incidence of this lesion was similar in control and treated rats. There was an increase in hepatocellular adenomas in treated female rats at the terminal sacrifice (18 in treated vs. 1 in controls).⁽⁷⁷⁾

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6.3.3. Interpretation

Interpretation of the Positive Study—Rats:

Toxicology. There was no weight depression in males fed 1% O-17 until 26–50 weeks. The chemical produced no weight depression in females. No effect on survival was found. Toxic hepatic lesions were seen in females but not in males.

Pathology. Liver tumors (hepatocellular adenoma or carcinoma) were found in increased incidence in females (21/59) fed 1% O-17 as compared with (3/52). The majority of these rats had hepatocellular adenomas while only 4 rats had carcinomas in the 1% group (one animal had both an adenoma and a carcinoma). Two carcinomas, however, were found in female controls. Mammary fibroadenomas were found in increased multiplicity and total numbers in 1% female rats but the incidence of these tumors was not significantly increased (34/59) when compared to controls (28/51).

Conclusion. The liver tumors and toxic liver lesions were found only in female rats. Based on lack of changes in body weight gain and survival these rats may have tolerated higher doses of O-17. The same effect may have been seen on the mammary tumors. However, the study is adequate.

O-17 was supplied by CTFA as FDA certified lot No. AA 6260 and listed as “97% pure.” No data were included on the further characterization of the purity of the color additive or the presence of impurities. Certified FDA lots should contain less than 0.2% of the starting materials; however, analysis of this lot showed 0.29% 2,4-dinitrobenzeneamine and 0.70% 2-naphthalenol when analyzed by the HPLC method.⁽²²⁾ The study seems to be well-controlled but lacks definitive information on the purity of the color and the levels of impurities that may be present.

Interpretation of the Equivocal Mouse Study:

Toxicology. Feeding of dietary levels of 1% of O-17 produced no depression in body weight gain. In the males, however, there was an effect of dose on survival. The cause of this increased mortality was not reported but toxic lesions were induced such as pigmentation in various tissues and myocarditis.

Pathology. There was an equivocal increase in liver tumors in high-dose male mice.

Conclusions. Increased mortality and toxic lesions were found in male mice, but this study does not provide definitive evidence for carcinogenicity in mice.

The chemical analysis of the lot of color was the same as that in the rat study since they were done together.

6.4. Skin Penetration Studies

The *in vitro* study⁽¹³⁾ used ¹⁴C-D&C Orange No. 17 obtained from Amersham, UK as 2,4-dinitro [U-¹⁴C]phenylazo-2-[1,4,5,8-(¹⁴C)]-naphthalenol with a reported specific activity of 5.6 mCi/mmol. The ¹⁴C label is distributed as shown in Fig. 7.

The supplier stated that the synthesis method used insures an equal contribution of the specific activity from each labeled part of the molecule. Data were supplied indicating that the radiochemical purity was 95 and 96% determined by TLC separation in two solvent systems. The presence of subsidiary colors or the identification of the remaining 4–5% of the radioactivity was not stated. Dose formulations were prepared in oils and cream blusher at 0.6% color and in talc at 5% color. Approximately 1.5×10^6 DPM of color was applied in oils, 4.7×10^6 in cream blusher, and 38×10^6 in talc. Volpo 20 was used in the receptor cell to aid solubilization of the pigment. Recoveries of 80–90% were seen in oil formulations and 40–50% in cream blusher. Recoveries were not addressed in the talc studies. Most of the dose was removed with toluene skin washes, although ¹⁴C was found in all skin preparations. Counting efficiencies were not stated.

This study used color formulations of defined radiochemical purity; however, the nature of the 4–5% impurity was not addressed. The question of solubility of the color in the receptor solution was adequately discussed. The rate of penetration through the skin was low. A concern can be raised that if the radioactive impurity was all 2,4-dinitrobenzeneamine (4–5% of the ¹⁴C), it might be preferentially absorbed by the skin. Since, however, skin absorption of ¹⁴C was less than 0.03% of the amount applied/day in liquid media and less than 0.001% of the amount

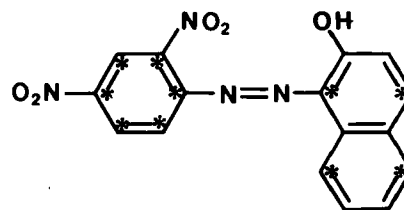


Fig. 7. Location of Radiolabel in major component of O-17.

applied/day in talc formulations, it is unlikely that this impurity would have a significant impact on risk assessment calculations because of the generally small amount of material that penetrates.

6.5. Specific Assumptions Used in the CTFA O-17 Risk Assessment

- *Assumption 1:* For creams and oils the absorption rate is 0.01%/day.

Validity: Unknown but testable. However, for insoluble materials, the interpretation of these rates is not simple.

Option: Conduct a live skin test for longer periods of time.

Impact: The skin test revealed rates of 0.003–0.007% of applied amount/24 hours, implying an overestimation of the absorption by a factor of 2. However, the real factor is unknown because it is not clear whether the above rates refer to a maximum rate or a steady-state rate. The Panel assumed an absorption rate of 0.005%/day of the amount applied, half that assumed by CTFA.

- *Assumption 2:* The absorption rate for O-17 in talc is 0.001%/day of the amount applied.

Validity: Probably correct and testable.

Options: Repeat *in vitro* skin studies with live skin models.

Impact: CTFA mentioned 0.001% absorption in its text,⁽³⁾ citing the skin penetrance study⁽¹³⁾ and printed 0.00009% in a table. The penetrance study uses the values 0.001%. The laboratory data sheets are difficult to interpret, therefore the Panel used 0.001%.

- *Assumption 3:* The use of Volpo 20 in the receptor of the Franz cell will not significantly alter the skin penetration.

Validity: Probably valid and testable. *In vivo* there are a number of factors, such as protein binding in blood flowing through the capillary bed, which would remove the color when it passed through the skin “solubilizing” the color, which was the reason for using the Volpo 20.

Option: Conduct parallel studies *in vivo* and *in vitro* using saline and 6% Volpo 20.

Impact: Use of Volpo 20 may result in higher observed skin penetrance rates. However, the effect on the exposure is not likely to be significant since total penetrance using Volpo 20 was extremely low. The Panel accepted use of the CTFA assumption.

6.6. Risk Characterization

6.6.1. CTFA Risk Estimates

From the BIO/DYNAMICS Study in CD-1 Mice. Data from an equivocal study were treated by the Panel as if it was positive, since it was done so in the CTFA risk assessment, and a comparison to the CTFA estimate was thought useful. Risk estimates were slightly higher using liver tumors (adenoma or carcinoma), rather than hepatocellular carcinoma only. Males were more sensitive than females.

From the BIO/DYNAMICS Studies in Charles River CD Rats. The risk estimate was based on liver tumors in females. Data from two very similar studies were combined to provide two dose levels plus controls.

6.6.2. Panel Risk Estimates

Application of the product- and color-specific assumptions to these usage data results in a total absorbed amount of color per day of 0.00189 µg/day as an average of the prospective survey; 0.00635 µg/day as the upper 90th percentile of the prospective survey. The comparable amount calculated by CTFA assumptions for the upper 90th percentiles is 0.0208 µg/day.

The factor 0.8 accounted for the effect of the general assumptions.

6.6.3. Comparison

See Table II.

Table II. Comparison of Risk Estimates of O-17^a

	Risk(CTFA/90) ^b	Risk/90	Risk/Rea
Rat	2.1×10^{-10}	5.1×10^{-11}	1.5×10^{-11}
Mouse	0.7×10^{-10}	1.7×10^{-11}	0.5×10^{-11}

^aNote that CTFA does not give an average exposure estimate for O-17.

^bRisk(CTFA/90) is the CTFA risk estimate at the upper 90th percentile of exposure.

^cRisk/90 is the risk estimate based on the Panel's calculation at the 90th percentile of exposure.

^dRisk(Rea) is the risk estimate based on the Panel's calculation of a more reasonable estimate of exposure.

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6.7. Summary

The animal studies were properly controlled except for a lack of data on the purity of the color (subsidiary color) or on the levels of impurities, at least one (2,4-dinitrobenzeneamine) of which is a potent mutagen. If this color were certified according to FDA specifications with the cellulose column chromatography method, the level of free amine should not exceed 0.2%. However, an HPLC method showed that the level of free amine was 0.29% in this lot. The HPLC method, with its more vigorous solubilization of "insoluble" color would result in the detection of higher levels of impurities than would be reported by the official certification method.

The level of impurities may have an impact on risk assessment only if the degree of potency of the impurities is substantially greater than that of the color. Based on the strong mutagenicity of O-17 without activation in several strains, the mutagenicity of the azo reduction product, and the reported mutagenicity of 2,4-dinitrobenzeneamine with or without activation, it is unlikely that the carcinogenic "potency" differs from the parent compound by several orders of magnitude.

It is important not to ignore the definite mutagenicity of the agent and its metabolite in bacteria. Because the chemical is only slightly soluble, one can speculate that the extent of absorption by the gut may be small; however, the role of intestinal bacterial enzyme activity, such as azo reductase on the azo bond, in producing soluble products should not be ignored. Possible slow absorption may be a factor in the weak carcinogenic response, and the risk assessment figures should be used cautiously with this genetically toxic agent.

7. D&C RED NO. 9 (R-9)

7.1. Chemistry

D&C Red No. 9 (CAS 5160-02-1) (CI 15585:1) (R-9) is a water-insoluble monoazo color additive which is the barium salt (2:1) of 5-chloro-2-[(2-hydroxy-1-naphthalenyl)azo]-4-methylbenzenesulfonic acid (FDA also terms it the barium salt of 1-(4-chloro-o-sulfo-5-tolylazo)-2-naphthalenol). It is commonly used as pure color and in a lake form. Its structure is shown in Fig. 8.⁽⁷⁸⁾

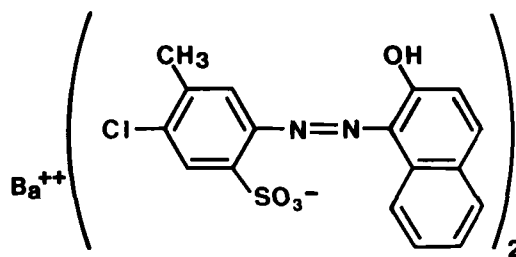


Fig. 8. Structure of major component of R-9.

R-9 is synthesized by diazotization of Red Lake C amine and coupling with 2-naphthalenol to produce D&C Red No. 8 (R-8). This color additive is then treated with barium chloride to produce the barium salt of R-8, known as R-9. The synthetic pathway is shown in Fig. 9.⁽²³⁾

FDA certification requires that each color additive lot meet the following specifications⁽⁴¹⁾:

- Volatile matter, not more than 5.0 percent.
- Ether extracts (isopropyl ether), not more than 0.5 percent.
- Red Lake C Amine, not more than 0.2 percent.
- Beta-naphthol, not more than 0.2 percent.
- Chlorides and sulfates of sodium, not more than 6.0 percent.
- Oxides of iron and aluminum, not more than 1.0 percent.
- Pure dye (as determined by titration with titanium trichloride), not less than 87.0 percent.

The methods of analysis for the two starting materials are for soluble impurities only and may underestimate the levels of impurities trapped within the crystal structure of this insoluble color.⁽²³⁾

FDA⁽²³⁾ describes the impurities likely to be present as Red Lake C Amine (2-amino-5-chloro-4-methylbenzenesulfonic acid) and its many possible isomers and impurities unique to each synthetic method, 2-naphthalenol, and the barium salt of 4-(4-chloro-2-sulfo-5-tolylazo)-1-naphthalenol (also termed 4-naphtholazobenzene-4-methyl-5-chloro-1-sulfonate) (a subsidiary color). Other nitroaromatics, subsidiary colors, by-products, and degradation products were also listed as possibly present. The Environ Report⁽⁶⁾ states that, in addition to the two starting materials, other likely impurities are 4 related subsidiary colors. Also, impure isomers of the starting amine may be present in small quantities. Unsulfonated aromatic amines could conceivably be present in isopropanol extractables, but at very low concentrations. Commercial color lots were shown to contain the starting materials and the subsidiary colors listed above.

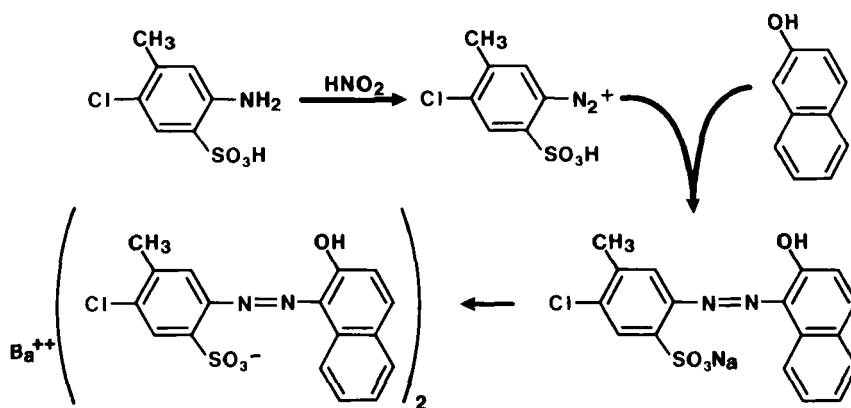


Fig. 9. Synthesis of major component of R-9.

Naganuma *et al.*⁽²⁹⁾ defined a good methodology to investigate the possible impurities in R-9, including positional isomers of the starting materials and the subsidiary colors produced from these isomers. The data show that the majority of the impurities present in R-9 can be attributed to the impurities present in Red Lake C Amine. Analysis of four lots of R-9 from US manufacturers showed that these amine impurities were present at 30–84 ppm. (Note: The colors analyzed by Naganuma were *not* used in the bioassays.) This paper also proposed a cost-effective method of synthesis of relatively pure Red Lake C Amine and R-9, scaled up for commercial use. The major change involves a purification of the Red Lake C Amine prior to diazotization and coupling with 2-naphthalenol. Other investigators reported 0.1–0.5% 4-(4-chloro-2-sulfo-5-tolylazo)-1-naphthalenol in three commercial samples of color from the US.⁽³⁰⁾

A supplemental report⁽³¹⁾ partially answered the questions of color additive purity (i.e., the presence of impurities, starting materials, and subsidiary colors) in the color lot used in the NCI rat bioassay⁽⁷⁹⁾ and the skin penetration study.⁽¹⁴⁾ An HPLC method was utilized to separate impurities using detection at 254 and 490 nm, in which a substance must absorb at both wavelengths to be detected. Thus, this method was only able to evaluate colored impurities.

The color lot used in the bioassay (FDA Certified lot no. Z8054, 89.8% color) was found to be 98.8% “pure” (area percent) with several other colored bands reported. Amine and non-azo impurities would not have been detected. The ^{14}C -labeled color used in the skin penetrance studies (Amersham-U- ^{14}C)-R-9) was also analyzed by the HPLC method and found to be 99.1% R-9 with 4 other colored bands. When analyzed for radiochemical purity,

95.6% of the radioactivity was associated with R-9, with eight other radioactive bands including Red Lake C Amine, and two other non-azo compounds.

This study was not designed to look at noncolored impurities; however, the color purity is better defined than by the nonchromatographic FDA certification method. One also questions why the data derived from a TLC method previously described⁽³¹⁾ were not presented. Other investigators⁽²⁹⁾ have done definitive work with TLC.

7.2. Metabolism

Two radiolabeled preparations have been synthesized; one labeled with ^{14}C in the benzene ring and the other labeled with ^{14}C in the naphthalenol ring. After administration of either preparation added to the diet in a concentration of 3000 ppm (0.3% of the diet) about 54 of the dose of the ^{14}C -naphthalenol label and about 35% of the dose of ^{14}C -benzene label were excreted into urine within 48 h.⁽⁸¹⁾ The compound is insoluble in water and there is no evidence that there would be a dose-dependent rate of dissolution of the compound in the gastrointestinal tract. Very little of the parent compound could be detected in the urine; instead, the radiolabel in the urine appeared to be associated predominantly with three metabolites. One of the metabolites containing the ^{14}C -naphthalenol label was tentatively identified as 1-amino-2-naphthalenol and the other as an unidentified conjugate. The principal metabolite containing the ^{14}C -benzene label was tentatively identified as Red Lake C Amine. Both metabolites would be expected on reduction of the azo-group. However, it is not clear whether the reduction occurred predominantly in the gastroin-

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testinal tract and the metabolites were then absorbed or whether the parent compound was absorbed and then eliminated in the liver predominantly by azo-reduction and biliary excretion. Unfortunately, the experiments were not designed to evaluate the two mechanisms or to determine the bioavailability of the compound.

Some of the ¹⁴C-naphthalenol label appears to be covalently bound to erythrocytes, possibly through the formation of a chemically reactive metabolite of the 1-amino-2-naphthalenol. Although the investigators suggest that the binding of the metabolite to erythrocytes might lead to the destruction of the erythrocytes in the spleen and thereby cause splenic injury, the hypothesis has not been adequately explored. Nevertheless, the fact that neither R-8 nor R-9 are mutagenic in bacterial systems (see below) suggests that the reactive metabolite formed from 1-amino-2-naphthalenol does not rapidly combine with DNA. This compound is not mutagenic in bacteria. However, a number of the colors which are reduced to this compound (such as O-17) are mutagenic, implying that the other amines such as 2,4-dinitrobenzeneamine generated may account for the mutagenicity of these other colors.

7.3. Short-Term Tests**7.3.1. Mutagenicity**

R-9 was negative in TA98, TA100, TA1535, and TA1537, with and without S9 activation,⁽⁶⁸⁾ and R-9 lake from another source was inactive in the same strains, plus TA1538.⁽⁴⁹⁾

7.3.2. DNA Damage/Repair

R-9 supplied by CTFA did not induce DNA synthesis over a wide dose range *in vitro* or after *in vivo* activation at high doses using hepatocytes.⁽⁸²⁾

7.3.3. Other

There were no data from chromosomal damage or transformation assays.

7.3.4. Significance

There is no evidence of mutagenicity in bacteria for this color or induction of DNA synthesis in

hepatocytes. Other systems have not been tested. There is no evidence for genetic toxicity of this color either by itself or as part of a lake.

7.4. Comparative Toxicology**7.4.1. Negative Studies**

Rat: R-9 was given at 0.01, 0.05, 0.25, and 1% of the diet to 25 male and 25 female Osborne-Mendel rats for two years. Although no oncogenic effect was noted, splenomegaly and other splenic lesions were noted, especially in rats on the two highest dose levels.⁽⁸³⁾

Groups of 60 male and female and then 70 male and female (2nd generation) Charles River SD rats received 0.01, 0.02, or 0.05% of R-9 in the diet in a two-generation study for a period up to 30 months.⁽⁸⁰⁾

In a second study on a similar protocol (the LBI study), 70 male and female rats were fed 1.0% of the color additive following *in utero* exposure. Splenic lesions were noted in the male rats fed 1% of R-9.⁽⁸⁰⁾

Mouse: In the NCI study, R-9 was given at 0.1 or 0.2% to groups of 50 male and 50 female B6C3F1 mice for 103 weeks. At the high dose there was a minimal change in body weight. No oncogenic effect was noted.⁽⁸⁴⁾

Charles River CD-1 mice (60 male and 60 females at each level) received 0.005, 0.025, or 0.1% of R-9 in the diet for 24 months. Neoplastic effects were not noted.⁽⁸⁰⁾

In a second study (the LBI study) 60 male and 60 female CD-1 mice received 0.2% of R-9 for 22 months. Neoplastic effects were not noted.⁽⁸⁰⁾

Skin Painting: In 50 male and 50 female ICR Swiss-Webster mice 0.1 ml of a 1% solution or suspension of R-9 was applied twice weekly to the skin for 18 months. No oncogenic effect was noted.⁽⁶⁷⁾

One-tenth ml of a 1% solution of R-9 was applied weekly to the skin of 50 male and 50 female CF-1 Carworth mice for 26 months. No oncogenic effect was noted.⁽⁷⁶⁾

7.4.2. Positive Study

Rat: In the NCI study, R-9 was given at 0.1 or 0.3% in the diet to 50 male and 50 female F344 rats in each group for 103 weeks. Females at the higher dose level showed a 6% weight depression compared to controls. Splenic sarcomas and neoplastic nodules of the liver were increased in male rats.⁽⁸⁴⁾

7.4.3. Interpretation

Interpretation of positive study:

Toxicology. R-9 in F344 rats produced no effect on survival when fed at dietary levels of 0.3%, but toxic lesions were found in the spleen of male and female rats. These toxic lesions have been described in rats fed diets containing aniline, azobenzene, dapsone, and other aromatic amines.⁽⁸⁵⁾

Pathology. Splenic sarcomas were found in 26/48 male rats fed 0.3% R-9 and in 0/50 control males. These were highly malignant, unusual tumors and several metastasized to peritoneal sites. None were seen in female rats fed R-9.

Hepatic neoplastic nodules were found in 7/49 high dose (0.3%) male rats and 6/50 low dose (0.1%) rats and 0/50 control males. High dose (0.3%) female rats had 5/50 nodules while controls had 1/50.

Conclusion. R-9 was carcinogenic in male F344 rats and also produced splenic toxic lesions associated with the induced splenic sarcomas. The mechanisms responsible for these tumors are unknown, but they are induced by some aromatic amines and related compounds.⁽⁶⁴⁾ All may produce methemoglobinemia. This study was adequate for determining the carcinogenicity of R-9 in F344 rats.

The rats were housed together in plastic-bottomed cages. R-9 was obtained from H. Kohnstamm & Co. as FDA certified lot Z-8054, 89.8% color. One peak was seen in an HPLC chromatogram. Other assays performed to characterize the color were elemental analysis, water content (1.58%), IR (consistent with standards), UV (no reference spectra), and TLC. Two TLC systems were used, one detected three impurities, the other 5 impurities. Detection was by visible and UV visualization. The nature of the impurities seen by TLC was not stated. Results from the FDA analysis showed 87% color, 1.8% volatile matter, 0.15% 2-naphthalenol, and less than 0.015% Red Lake C Amine.⁽¹⁰⁸⁾ The dosing seemed to be well-controlled.

One criticism of this study was the group housing of the rats in solid-bottom cages where rats could ingest feces likely to contain the metabolites of the color produced by the azo-reductases of the GI tract. Some of these aromatic amines are established human carcinogens, others are suspect human carcinogens. The amount of coprophagy was likely to be small as animals had access to feed and water at all times. Nevertheless, there is the possibility that potentially carcinogenic aromatic amine metabolites could have been ingested.

7.5. Skin Penetration Studies

The *in vitro* studies on R-9⁽¹⁴⁾ used ¹⁴C-R-9 obtained from Amersham, UK, labeled on the “benzene” ring, presumably on the Red Lake C Amine ring. The stated specific activity was 10.6 mCi/mmol. No data were supplied in the penetrance study on the radiochemical purity; however, the supplemental report showed a radiochemical purity of 95.6%, with 8 other radioactive fractions seen, including Red Lake C Amine and two other non-azo compounds.⁽³¹⁾

Formulations were prepared in oils and talc at 5% color and dosed as 3 μ Ci/5 μ l/cm². A cream blush formulation was prepared at 0.5% and dosed at 1.5 μ Ci/25 μ l/cm². Approximately 3–4 million DPM of ¹⁴C was applied in each preparation. Volpo 20 was used in receptor cells to aid the solubility of the color. Most of the material was found in skin washings with good overall recovery of ¹⁴C. Counting efficiencies were less than 30% because of the large amount of aqueous fraction counted in the gelled counting cocktail.

The experimental design seems sound with the exception of the lack of information on radiochemical purity and impurity levels, partially addressed by the supplemental report. Characterization of the identity of the separated impurities would have aided risk assessment deliberations.

7.6. Specific Assumptions Used in the CTFA R-9 Risk Assessment

- **Assumption 1:** The absorption rate for R-9 is 0.06%/day of the applied amount, except for those products that need adjustment (e.g., nail polish).

Validity: Would be reasonable if the above rate is a steady-state absorption rate. Although the penetrance study report⁽¹⁴⁾ indicated that no steady state was reached, the data indicate a plateau for split-thickness skin chambers with castor oil and cream as vehicles—hence, a steady-state rate. The data provided indicate that absorption values ranging from 0.016 to 0.064%/day were reached after 2 days. The Panel accepted use of CTFA’s estimate.

- **Assumption 2:** The use of Volpo 20 in the receptor of the Franz cell will not significantly alter the skin penetration.

Validity: Probably valid and testable. *In vivo* there are a number of factors, such as protein binding in blood flowing through the capillary bed, which would remove the color when it passed through the

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skin, and thereby “solubilizing” the color that penetrates.

Option: Conduct parallel studies *in vivo* and *in vitro* using saline and 6% Volpo 20.

Impact: Use of Volpo 20 may result in higher observed skin penetrance rates. The effect is not likely to be significant for the risk estimate. The Panel used this assumption.

- *Assumption 3:* 50% of the amount of lipstick applied will be swallowed.

Validity: Unknown but testable.

Option: Conduct a study in women on the amount of lipstick swallowed using a radiochemically labeled color or other tracer. Include measurement of the amount lost due to contact of the lips with other surfaces or to wiping off.

Impact: The Panel agrees with CTFA that 50% is likely to be an overestimation. Since specific data for adjusting the percentage swallowed are not available, however, the Panel accepted use of CTFA's estimate.

7.7. Risk Characterization

7.7.1. CTFA Risk Estimates

From the NCI rat study: The most sensitive carcinogenic end-point was splenic tumors in males.

7.7.2 Panel Risk Estimates

Information on the usage frequency indicates that R-9 is present in 6 groups of products. Application of the color- and product-specific assumptions to the usage data results in a total absorbed amount of:

- 1.211 $\mu\text{g}/\text{day}$ as an average of the retrospective usage survey;
- 1.716 $\mu\text{g}/\text{day}$ as the upper 90th percentile of the retrospective survey, as compared to the CTFA estimate of 3.44 $\mu\text{g}/\text{day}$.

For ingestion, the use of the color- and product-specific assumptions and the values of application frequency and amount per application given by CTFA leads to an ingested amount:

- 86 $\mu\text{g}/\text{day}$ as an average based on a survey of ingested lipstick;
- 415 $\mu\text{g}/\text{day}$ as the upper 90th percentile

Both figures are similar to CTFA.

Table III. Comparison of Risk Estimates for R-9^a

	Risk(CTFA/90) ^b	Risk/90 ^c	Risk/Rea ^d
External	4.3×10^{-8}	1.7×10^{-8}	1.2×10^{-8}
Ingested	5.1×10^{-6}	4.3×10^{-6}	8.9×10^{-7}

^aNote, CTFA did not calculate risk estimates based on average exposure levels.

^bRisk(CTFA/90) is the CTFA risk estimate at the upper 90th percentile of exposure.

^cRisk/90 is the risk estimate based on the Panel's calculation at the 90th percentile of exposure.

^dRisk(Rea) is the risk estimate based on the Panel's calculation of a more reasonable estimate of exposure.

As discussed earlier, it is believed that the prospective survey is less biased than the retrospective study. No results, however, have been provided from the prospective survey so the retrospective study data are used. Appropriate factors 0.8 and 6/7 were used to account for the general assumptions.

7.7.3. Comparison

As shown in Table III, the risk from ingestion does not include the risk due to absorption of R-9 through the lip surface. Since the skin absorption rate of any of the tested vehicles was less than 0.1%, the incremental risk from absorption through the lip surface would be orders of magnitude lower than the risk from ingestion.

7.8. Summary

The NCI study is complicated by the possible ingestion of fecal metabolites. The analyses of color do not define the levels of non-azo impurities. Both effects are not likely to produce large changes in the risk assessment calculations.

For the skin studies 95.6% of the radioactivity was associated with the R-9 peak, with 8 minor peaks including some non-azo material and some starting material. The radiochemical purity seems to be sufficient so that the risk assessment calculations are not significantly altered.

As a final word of caution, the risk estimates are based on a recent usage survey and any increase or decrease in the color usage will result in proportionate changes in the estimate of risk. Also, it is merely a CTFA recommendation to limit the dye to 2% in lipstick as it is used in the CTFA risk assessment (FDA regulations allow 3%). It is not binding, however, and exceeding it could lead to an increase

in the risk (however, because of the insolubility of the compound, especially as a lake, an increase in concentration may be meaningless).

8. D&C RED NO. 8 (R-8)

8.1. Chemistry

D&C Red No. 8 (CAS 2092-56-0) (CI 15585) (R-8) is a sparingly soluble monoazo color additive which is the monosodium salt of 5-chloro-2-[(hydroxy-1-naphthalenyl)azo]-4-methylbenzenesulfonic acid (FDA also terms it the monosodium salt of 1-(4-chloro-o-sulfo-5-tolylazo)-2-naphthalenol). It is commonly used as pure color and as lakes. Its structure is shown in Fig. 10.⁽⁸⁷⁾ It is the sodium salt of R-9.

R-8 is synthesized by diazotization of Red Lake C amine and coupling with 2-naphthalenol. The synthetic pathway is identical to that shown above for R-9 except for the last step.⁽²³⁾

FDA certification requires that each color additive lot meet the following specifications⁽⁴¹⁾:

- Volatile matter, not more than 10.0 percent.
- Ether extracts (isopropyl ether), not more than 0.5 percent.
- Red Lake C Amine, not more than 0.2 percent.
- Beta-naphthol, not more than 0.2 percent.
- Chlorides and sulfates of sodium, not more than 5.0 percent.
- Mixed oxides, not more than 1.0 percent.
- Pure dye (as determined by titration with titanium trichloride), not less than 85.0 percent.

FDA⁽²³⁾ describes the impurities likely to be present as Red Lake C Amine (2-amino-5-chloro-4-methylbenzenesulfonic acid) and its many possible isomers and impurities unique to each synthetic method, 2-naphthalenol, and 4-(4-chloro-2-sulfo-5-tolylazo)-1-naphthalenol (a subsidiary color). A wide variety of nitroaromatics, possibly subsidiary colors, by-products, and degradation products were also listed as possibly present. The Environ Report⁽⁶⁾ does not

address R-8, but impurities could be expected to be similar to those described for R-9. Commercial color lots were shown to contain the starting materials and the subsidiary color listed above.

8.2. Short-Term Tests

8.2.1. Mutagenicity

In addition to the work reported for R-9, R-8 itself was negative in TA98, TA100, TA1535, and TA1537, with and without S9 activation.⁽⁴⁶⁾ R-8 supplied by FDA was also negative in TA98 as was the color as part of a lipstick preparation.⁽⁴³⁾

8.2.2. Other

There were no reports for DNA damage/repair, chromosomal damage or transformation.

8.2.3. Significance

The effects of this color in short-term tests are expected to be similar to R-9, since the only difference is the cation. There is no evidence for mutagenicity in bacteria for R-8. Other systems have not been tested. There is no evidence for genetic toxicity of this color, either by itself or as part of a preparation.

8.3. Comparative Toxicology

No long-term animal assays have been performed with this color additive. However, there is little reason to suspect that the material would be different toxicologically from R-9 once it was absorbed. When the color reaches the stomach, it is highly unlikely it is maintained as the sodium salt, and R-8 and R-9 are likely to be similar, if not identical, ionic forms in the gut.

8.4. Skin Penetration Studies

Skin penetration studies have not been performed with this color additive. The increased water solubility may increase skin penetrance of R-8 compared to R-9 when both are used as pure color. However, when both color additives are used as lakes, there is not likely to be any difference in

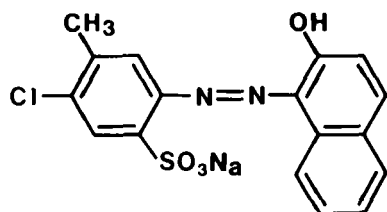


Fig. 10. Structure of major component of R-8.

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solubility and, thus, skin penetration would be expected to be nearly identical.

8.5. Specific Assumptions Used in R-8 Risk Assessment

- *Assumption 1:* The information on R-9 can be used to predict the risk from R-8.

Validity: Reasonable and testable. The risk assessment performed on the closely related color additive, R-9, may have some relevance to R-8. R-8 is a sodium salt and is more soluble than R-9. There is no R-8 exposure information.

Option: Conduct testing on the toxicity and absorption of R-8.

Impact: The increased solubility may increase penetrance.

8.6. Risk Characterization

8.6.1. CTFA Risk Estimates

CTFA made no estimates of risk from this color.

8.6.2. Panel Unit Risk Estimates

R-9 is a barium salt and R-8 is the sodium salt of the same anion. Given the highly acid milieu of the stomach, there would be no reason for assuming that R-8 would behave differently from R-9 with regard to the cancer response to oral dosing. Once absorbed, therefore, it is reasonable to assume an equal toxicity and an equal dose-response curve.

With regard to absorption through the skin there are differences between the two colors with regard to their solubility in water. However, it remains unclear whether such differences would have any impact on the absorption rate if R-8 would have been tested at prevailing concentrations in vehicles compatible with marketed products.

It must, however, be understood that there is no exposure information for this color, and the unit risk estimate is based on simple extrapolation from a chemically related color.

Thus, the nature of this risk estimate is different from those calculated for R-19, O-17, R-9, and R-3. This risk estimate is actually a unit risk, similar to R-37, of 4.3×10^{-8} for an absorbed dose of 3.44 $\mu\text{g}/\text{day}$ to R-9 type colors. There is no information on actual human exposure, except, based on produc-

tion data of the colors, that it is probably less than R-9.

8.7. Summary

The few tests for genetic toxicity that have been performed have been negative. Since it is reasonable to assume that R-8 has the same toxicological effects as R-9, any difference in the risk is probably related to any differences in exposure. The unit risk estimates should be used whenever exposure estimates are available to calculate absolute risk.

9. FD&C RED NO. 3 (R-3)

9.1. Chemistry

FD&C Red No. 3 (CAS 16423-68-0) (CI 45430) (R-3) is a water soluble xanthene dye 3',6'-dihydroxy-2',4',5',7'-tetraiodo-spiro[isobenzofuran-1(3H), 9'-[9H]xanthene]-3-one, disodium salt, (known also by the FDA as "principally the monohydrate of 9(o-carboxyphenyl)-6-hydroxy-2,4,5,7-tetraiodo-3H-xanthene-3-one, disodium salt, with smaller amounts of lower iodinated fluoresceins"). Its common name is erythrosine and its structure is shown in Fig. 11.⁽²⁴⁾

R-3 is synthesized by the condensation of phthalic anhydride and resorcinol. The intermediate product 2-(2',4'-dihydroxybenzoyl)benzoic acid is condensed with another mole of resorcinol to produce fluorescein. The fluorescein is then iodinated to produce R-3. The synthetic pathway is shown in Fig. 12.

FDA certification requires that each color additive lot meet the following specifications⁽⁴¹⁾:

Volatile matter, and chlorides and sulfates (calculated as the sodium salts), total not more than 13 percent.
Water-insoluble matter, not more than 0.2 percent.

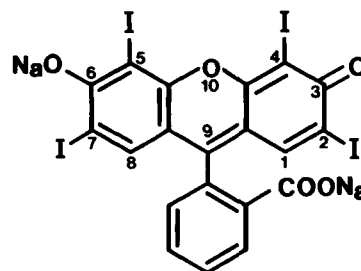


Fig. 11. Structure of major component of R-3.

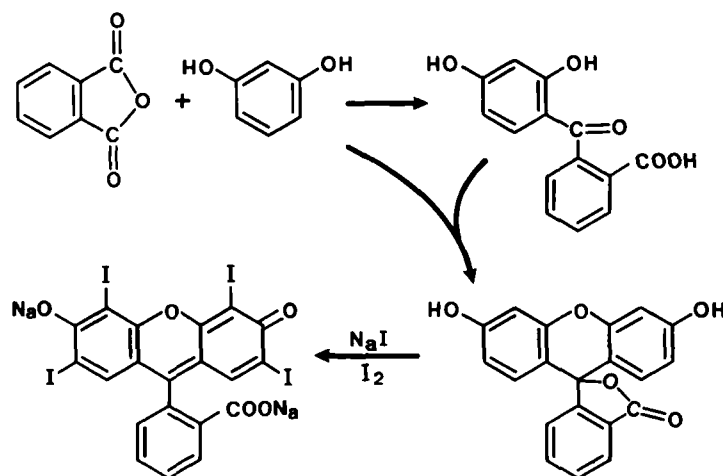


Fig. 12. Synthetic pathway of major component of R-3.

Unhalogenated intermediates, total not more than 0.1 percent.

Triiodoresorcinol, not more than 0.2 percent.

2-(2',4'-Dihydroxy-3',5'-diiodobenzoyl)benzoic acid, not more than 0.2 percent.

Other lower iodinated fluoresceins, not more than 9 percent.

Lead (as Pb), not more than 10 parts per million.

Arsenic (as As), not more than 3 parts per million.

Total color, not less than 87 percent.

The starting materials and intermediates are possible impurities as well as triiodinated fluorescein. Two reports^(6,24) suggest that the following impurities and subsidiary colors may be present: phthalic acid, phthalic anhydride, resorcinol, 2-(2',4'-dihydroxybenzoyl)benzoic acid, iodine, sodium iodide, 2-(2,4-dihydroxy-3',5'-diiodobenzoyl)benzoic acid, triiodoresorcinol, 2,4,7-triiodofluorescein, 2,4,5-triiodofluorescein, 4,5-diiodofluorescein, 2,5-diiodofluorescein, 4-iodofluorescein, 2-iodofluorescein, and fluorescein. Other impurities are possible, depending on the purity of the starting materials. HPLC chromatograms obtained from FDA showed the separation of subsidiary colors at 436 and 546 nm.⁽²⁵⁾ Of the 5–8 peaks seen, R-3 was always the predominant peak with 2,4,5-triiodofluorescein the predominant subsidiary color. Other subsidiary colors identified in “commercial” lots were 2,4,7-triiodofluorescein, 2,7-diiodofluorescein, and a series of small peaks identified as “group.” In the chromatograms examined, R-3 was present at 90–94% of the colors detected at the two visible wavelengths. Two reports examined “Erythrosin B” and “Erythrosine” for the presence of subsidiary colors and impurities. Commercial sam-

ples of “Erythrosin B” from eight suppliers in the UK were examined using TLC and were found to contain numerous colored bands (subsidiary colors) as well as several bands detected with 254 nm UV light. Major subsidiary colors identified were 2,4,5-triiodofluorescein, 2,4,5-triiodo-4',5',6',7'-tetrachlorofluorescein, 4,5-diiodofluorescein, and 4-iodofluorescein. None of the UV absorbing bands were identified.⁽³²⁾ Three samples of commercial “Erythrosine” from Japanese suppliers, were examined by HPLC using reverse-phase chromatography and detection at 230 nm. The results showed the absence of starting materials, except for traces of phthalic acid in one lot, and the presence of 2–3 unidentified subsidiary colors.⁽²⁷⁾ No quantitative data or definitive analyses of impurities were given.

9.2. Metabolism

After intravenous administration to rats, R-3 is rapidly eliminated predominantly into bile. The clearance by way of the bile was about 55 times that into urine. Ultimately, most of the compound is excreted into the feces. At one time it was thought that high protein bound iodine in blood plasma might reflect the metabolism of the color by a deiodination reaction, but further study revealed that the substance was the color rather than thyroxine.⁽⁸⁸⁾ Although there is no definitive evidence that the substance is converted to other metabolites, the possibility that small amounts of the compound undergo metabolism can never be completely excluded. Nevertheless, the finding that R-3 is not a mutagen in

bacteria may support the view that it is not converted to chemically reactive metabolites that react with DNA, but certainly does not preclude such reactions in other systems. Since the predominant route of elimination is the bile, much of the compound absorbed from the gastrointestinal tract may be returned to the intestine by way of the bile before it enters the systemic circulation. The finding that substantial amounts of the unchanged substance are excreted into feces is not necessarily indicative of dose-dependent absorption. The data are not sufficiently complete, however, to estimate hepatic extraction ratios and, thus, the relative steady-state concentrations in the gastrointestinal tract, the liver, the systemic blood, and extrahepatic organs remain unknown. Nevertheless, we may anticipate that if the compound is as readily excreted in bile of humans, as it is in rats, that the largest area under the curve (*AUC*) of the aqueous concentration of the compound used as a cosmetic will be in the skin. The aqueous phase of the cells in the thyroid should be among the compartments having the lowest *AUC* values for the aqueous concentrations of the compounds.

9.3. Short-Term Tests

9.3.1. Mutagenicity

In *Salmonella* TA98 and TA100 (with or without activation with microsomal mix or light), color obtained from the National Institute of Hygienic Sciences (Japan) showed no mutational activity.⁽⁸⁹⁾ Color obtained from a number of other suppliers was also without effect, over a broad dose range, both in these assays^(43,90-93) and in fluctuation analyses using TA1538.^(94,95) Certified food color and R-3 from Allied Chemical Co. (93% "pure") was also negative for TA98, TA100, TA1537, and TA1538.^(96,97) There was one report that the color formulated by Durand and Huguenin in Switzerland is active in TA100 (an activity that disappears with S9 activation), but not in TA98, TA1535, and TA1538.⁽⁹⁸⁾ In *E. coli*, although there is some evidence of an effect in reversion of the pro locus,⁽⁹⁹⁾ in WP2 strain there is no effect either in the trp locus^(89,90) or when using a fluctuation analysis.^(94,95)

In eukaryotes, *S. cerevisiae* D7 and XV185-14C (a haploid) strains showed a significant effect in one locus with a dose response.⁽¹⁰⁰⁾ However, there was no significant induction of *S. chevalieri* respiratory-

deficient mutants over a wide dose range using erythrosine from two different suppliers. In fact, there was an inhibition of mutation induced by 4-nitroquinoline-1-oxide.⁽¹⁰¹⁾ Gene conversion did not occur in BZ 34 strain, either in resting or log phase under conditions of the test (4-h exposure at 5mg/ml).⁽¹⁰²⁾ Gene conversion did occur in the D7 yeast strain over a wide range of doses after a 24-h exposure (100), although there was no effect on intergenic mitotic recombination in D5 strain in the same experiment. There was no effect in inducing lethal *Drosophila* mutations or in onion root tip under conditions of light or dark, although there appeared to be an effect in *Vicia faba* in the light.⁽⁵⁶⁾

Thus, there appears to be little evidence for a mutagenic effect of the color in bacteria or in higher organisms except yeast and *Vicia faba*. Interpretation of the one report of an effect in TA100 is complicated by the lack of response in other, similar strains over a wide range of doses in the same experiment and may be a result of impurities in the particular sample or some experimental factor. In yeast, only some strains appear to be sensitive. The reasons for the differential sensitivity may be related to the different endpoints evaluated and the length of exposure to the agent. Also, the authors suggest that the longer growth period used in the D5 mitotic recombination assay may have selected against the expression of a genetic effect resulting from a short exposure time⁽⁹⁹⁾; however, there is little support for this contention. The results in *Vicia faba* may be induced by chemical toxicity since R-3 is phototoxic.⁽⁵⁴⁾

9.3.2. DNA Damage/Repair

Although originally found to be negative in a rec assay,⁽⁵¹⁾ which involves damage repairable by a particular enzyme system, the color was later found to give a positive response if the cultures were exposed to light.^(89,103,104) R-3 from another source, prepared to commercial standards, gave a negative response⁽⁹⁵⁾ with or without light exposure or microsomal activation at a lower dose. In leucocyte cultures exposed to light, there was an inhibition of DNA synthesis.⁽¹⁰⁵⁾ In yeast, the results in D7 showing gene conversion, which may be related to a recombination repair, are difficult to evaluate, as indicated above, especially with the negative results in strain D5. Using hepatocytes, over a wide dose range *in vitro* and at a high dose *in vivo*, the color did not induce any DNA repair.⁽⁸²⁾

There appears to be an effect of light and source on the action of this color in solution of inducing DNA damage. The effect of source may be related to the presence of impurities in different formulations. This would be consistent with the results seen in light since storage of the solution in liquid phase for some time by the manufacturer could result in generating active species. The results in these assays should be approached with caution for extrapolation to other biological endpoints. 10^{-4}M is the LC_{50} for human skin cell lines⁽⁵⁴⁾ and fetal rat hepatocytes,⁽¹⁰⁶⁾ with the toxic effect in culture suggested to be related to the consequences of dehalogenation.⁽⁵⁴⁾

9.3.3. Chromosomal Damage

In vitro there was no increase in chromosomal aberrations in human lymphocytes⁽¹⁰⁵⁾ (although there was a decrease in DNA synthesis) nor mitotic crossing-over in D5 yeast.⁽¹⁰⁰⁾ There was an increase in chromatid alterations in Ehrlich ascites cells⁽⁵⁶⁾ at doses toxic to human cells (although the authors considered the changes mutagenic, there was no evidence the changes were heritable). Positive results in the micronucleus test were reported as evidence of a clastogenic effect in male Swiss mice⁽¹⁰⁷⁾; however, no significant effect was seen in an experiment with better controls in Balb/C mice.⁽⁹²⁾

Again the question of toxicity complicates interpretation, especially for unusual tests. Dying cells release compounds which have complex effects on surrounding cells. It is not clear that the chromosomal effects on cells are a result of direct action on the genome.

9.3.4. Transformation

In a transformation assay in Fischer rat embryo cells, no effect was seen.⁽¹⁰⁸⁾

9.3.5. Significance

In sum, it appears that R-3 is not mutagenic in bacteria, except possibly in a single *E. coli* strain, or in higher organisms, except in some strains of yeast or in *Vicia faba*. The mutagenesis is clearly shown for haploid yeast, and is weak compared to ethyl methanesulfonate. From these results, it also appears that any effect is more related to DNA transitions rather than frameshift mutations. The color is positive in the rec assay after light activation and it affects DNA synthesis. There are chromosomal ef-

fects in Ehrlich tumor cells and *Vicia faba*, but not in human lymphocytes. The results in the micronucleus tests, as a whole, show no consistent effect.

The significance of the short-term tests for prediction of long-term toxicity is not straightforward. Except for one test done before modern standardization, the bacterial results for mutation are negative, with toxicity appearing to be a major factor in the positive response in the recombination assays. In yeast, only some strains and endpoints are positive.⁽¹⁰⁹⁾ The haploid reversion mutation assay appears to have an accuracy of approximately 0.6 in predicting carcinogenicity.⁽¹¹⁰⁾ The yeast mutation assays are not conducted according to an agreed-upon protocol and tend to be redundant to the *Salmonella* test, while the conversion assays are not sufficiently consistent and reproducible between laboratories.⁽¹¹¹⁾ The Ehrlich tumor cell assay is hard to interpret because of the instability of the tumor genome. The *Vicia faba*⁽¹¹²⁾ assays require much more development to be predictive of long-term health effects.

The short-term test results for R-3 were evaluated using a recent approach,⁽¹¹³⁾ which predict a probability of carcinogenicity for the color of 0.12 to 1.7%, a value similar to that of sucrose.⁽¹¹⁴⁾ The results suggest that the color is almost assuredly not a carcinogen acting directly on the genome.

The question of how the toxic effects of the compound, especially the phototoxic effect and the release of iodine, are involved in some of the short-term effects seen is a critical, unanswered one. The effect of toxicity in the assays for genetic damage should be clearly separated from a direct effect on the genome, which is difficult to do for many of these observations since the exposures which are effective seem to be toxic ones.

9.4. Comparative Toxicology

9.4.1. Negative Studies

Rat: R-3 was given at 4% of the diet to a total of 10 male and female Wistar rats for 2 years. One case of hepatic cirrhosis was noted.⁽⁶⁰⁾

R-3 was given at 0.5, 1.0, 2.0, or 5.0% of the diet to groups of 12 male and 12 female Osborne-Mendel rats for 2 years. Growth depression was noted in rats on the 5% level. Spleen weights were decreased. No neoplasms were attributed to the treatment.⁽¹¹⁵⁾

Subcutaneous injections of R-3 solutions (total dose = 12 mg/rat) in 9 male and 9 female rats yielded no tumors at the injection site after 2 years.⁽¹¹⁵⁾

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R-3 was given at 0.1, 0.5, and 1% of the diet to groups of 70 male and 70 female Charles River rats in a two-generation study which lasted for 24 months. No oncogenic effect was noted.⁽¹¹⁶⁾

R-3 was given at 0.25, 1, and 4% of the diet to groups of 25 male and 25 female CD rats (at each level) in a three-generation study. There were decreases in body weights of some of the treated groups (4% level). No compound-related effects were noted in the F_{3b} generation.⁽¹¹⁷⁾

Mouse: R-3 was given at 0.3, 1, and 3% of the diet for 24 months to 60 male and 60 female Charles River CD mice in each group. Body weights were decreased slightly at the 3% level. No oncogenic effect was noted.⁽¹¹⁸⁾

Gerbil: R-3 was fed to groups of 15 or 16 Mongolian gerbils at 1, 2, or 4% of the diet for 105 weeks. In addition, groups of 20–26 male and female gerbils were intubated twice weekly with 200, 750, or 900 mg/kg of R-3 by stomach tube for 97 weeks. Enlargement of the thyroids occurred in animals on the 1–4% dietary levels but neoplasms were not noted.⁽¹¹⁹⁾

Dog: R-3 was fed to groups of 3 male and 3 female beagle dogs at 0.5, 1.0, or 2% of the diet for 2 years. No effects due to ingestion of the color additive were seen.⁽¹¹⁵⁾

Skin Painting Study: In 50 male and 50 female ICR Swiss-Webster mice, 0.1 ml of a 1% solution of R-3 was painted on skin twice weekly for 18 months. No oncogenic effect was noted.⁽⁶⁷⁾

9.4.2. Positive Study

Rat: R-3 was given at 4% of the diet for 24 months to 60 male and 60 female (F₀) and 70 male and 70 female (F₁) CD rats which had been exposed *in utero*. Body weights of treated rats were lower than those of controls. Thyroid weights were increased in treated F₁ males, and the incidence of thyroid follicular tumors was increased.⁽¹²⁰⁾

9.4.3. Interpretation of Positive Rat Study

Toxicology. At the highest dietary dose level (4%), R-3 caused moderate body weight gain depression as compared with controls. There was no effect on mortality. Toxic lesions were seen in the thyroid glands of male F₁ rats which appeared to represent a goitrogenic effect of R-3 on the thyroid gland. These doses led to increased thyroid weights in males.

Pathology. Thyroid tumors (follicular adenoma or carcinoma) were found in 18/70 4% males and in only 1/70 control males. Only 3 of 18 rats with thyroid follicular cell tumors had a carcinoma of these cells.

Conclusion. Although R-3 at 4% of the diet with *in utero* exposure induced some weight depression, only a relatively low incidence of induced thyroid tumors was seen. Thyroid toxic lesions were also relatively mild and mortality was not affected. The study does show that R-3 is a goitrogen and thyroid carcinogen in rats.

R-3 was obtained from CTFA as FDA certified lot no. AA 2459 (733 Erythrosin), with a stated color content of 90% (dark red powder). No data were provided in the bioassay report on the presence of impurities or subsidiary colors that may have been present. FDA certification analyses of this lot show 90% color, 3.6% volatile matter, 7% inorganic salts, 0.19% sodium iodide, 0.07% insoluble matter, and nondetectable levels of phthalic acid, 2-(2',4'-dihydroxy-3',5'-diiodobenzoyl)benzoic acid and ether extractables. Analyses of subsidiary colors showed 4.66% 2,4,5-triiodofluorescein, 0.33% 2,4,7-triiodofluorescein, 0.2% "x", and 94.8% R-3.⁽²⁶⁾ The dosing in the study seemed to be well-controlled.

The purity of the color dosed is not stated in definitive terms. All that is shown is that the color was an FDA certified lot of "90% purity." No impurities were reported, however, the FDA certification analyses showed that the principal impurity was 2,4,5-triiodofluorescein. It is unlikely that significant amounts of identified impurities were present to the extent that the risk assessment would be significantly altered.

9.5. Skin Penetration Studies

The *in vitro* studies⁽¹⁵⁾ used ¹⁴C-R-3 obtained from the Pathfinder Laboratories, with a specific activity of 4.53 mCi/mmol. Although the report from Pathfinder assured the radiochemical purity of the labeled color,⁽¹²¹⁾ the data, an HPLC tracing with detection at 254 nm, demonstrated only that the material synthesized was, indeed, R-3. These data were totally inadequate to determine whether the radioactive label was actually incorporated in the material termed R-3. There is the possibility of uncolored ¹⁴C-labeled starting materials. In light of this, the skin penetration studies are impossible to interpret since it is not certain that the radiolabeled

material that penetrated the skin was R-3. There are also concerns about the low counting efficiencies reported, and the lack of skin coloration in the presence of significant ^{14}C , as the author of the study stated when interviewed.⁽¹²²⁾

Therefore, there is no adequate study of skin penetration of the color.

9.6. Specific Assumptions Used in the CTFA R-3 Risk Assessment

● *Assumption 1:* For aqueous or ethanol/water solutions, and oil/water emulsions, the absorption rate is 1%, and for talc 0.002% (i.e., the R-19 data) of the applied amount of color, except for those products that need adjustment.

Validity: Unknown and simple and easy to test. Since there is no adequate study available to evaluate this assumption, use was made of the data available for R-19, another xanthene, water-soluble color, as a model for R-3.

Option: Either: (a) Conduct *in vivo* tests for extended periods of time; or (b) Repeat the *in vitro* study with live-skin chambers and for extended periods of time with identified material.

Impact: As a larger molecule, it is unlikely that R-3 penetrates the skin as well as R-19; therefore, the absorption estimation based on R-19 probably overestimates the exposure. The Panel used the R-19 penetrance data.

● *Assumption 2:* 50% of the amount of lipstick applied will be swallowed.

Validity: Unknown but testable.

Option: Conduct a study in women on the amount of lipstick swallowed using a radiochemically labeled color or other tracer. Include measurement of the amount lost due to contact of the lips with other surfaces or to wiping off.

Impact: The Panel agrees with CTFA that 50% is likely to be an overestimation. Since specific data for adjusting the percentage swallowed are not available, the Panel used CTFA's estimate. However, CTFA's estimate of 14.5 mg lipstick per application $\times 2.86$ applications per day $\times 1\%$ concentration $\times 50\%$ swallowed results in 3.9 $\mu\text{g}/\text{kg}\cdot\text{day}$; not in 5.5 $\mu\text{g}/\text{kg}\cdot\text{day}$ as stated in the risk assessment.⁽⁵⁾

9.7. Risk Characterization

9.7.1. CTFA Risk Estimates

From data for thyroid follicular tumors in male Charles River CD rats: The data were combined for the two IRDC studies. Due to an interim sacrifice at one year, and since no thyroid tumors were observed before one year, the number of animals at risk was taken to be the number of survivors at one year.

9.7.2. Panel Risk Estimates

Information on the usage frequency of products containing R-3 indicates that five product categories belong to products with an assumed 1% absorption per day, three categories are talc-based products, and two are bath products. Application of the above two groups of product- and color-specific assumptions to these in the CTFA risk assessment report⁽⁵⁾ results in a total absorbed amount of color per person per day of:

0.88 $\mu\text{g}/\text{day}$ = 0.0166 $\mu\text{g}/\text{kg}\cdot\text{day}$ as an average of the prospective survey;

0.9016 $\mu\text{g}/\text{day}$ = 0.017 $\mu\text{g}/\text{kg}\cdot\text{day}$ as the upper 90th percentile of the prospective survey;

Table IV. Comparison of Risk Estimates for R-3

	Risk(CTFA/90) ^a	Risk(CTFA/Avg)	Risk/90 ^b	Risk/Rea ^c
External	3.2×10^{-8}	1.9×10^{-8}	2.0×10^{-9}	2.0×10^{-9}
Ingested	8.1×10^{-7}	NR ^d	4.9×10^{-7}	0.1×10^{-8}

^aRisk(CTFA/90) is the CTFA risk estimate at the upper 90th percentile of exposure.

^bRisk/90 is the risk estimate based on the Panel's calculation at the 90th percentile of exposure.

^cRisk(Rea) is the risk estimate based on the Panel's calculation of a more reasonable estimate of exposure.

^dNR-Not reported.

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The comparable CTFA's estimates were 6.650 and 11.3 $\mu\text{g}/\text{day}$, respectively. In lip products, the daily absorbed dose is 0.20 mg/day or 3.9 $\mu\text{g}/\text{kg}\cdot\text{day}$ for the 90th percentile. The average dose, according to a survey of lipstick ingestion, is 0.038 mg/day. Appropriate factors of 0.8 and 6/7 were used to adjust for the effect of the general assumptions.

9.7.3. Comparison

The major exposure differences between the CTFA (see Table IV) and Panel estimates derive from exposure from talc products, which is much less when a lower absorption is assumed. For this color, unlike R-19, O-17, and R-9, there was no adequate study of skin penetrance, so the absorption of R-19, a color in the same chemical class, was used. Since this color is a larger molecule, this model probably overestimates penetrance.

9.8. Summary

The color purity of 90% by FDA certification in the long-term assays does not adequately define the nature of the total color content. The amounts of organic impurities and subsidiary colors are defined by FDA certification methods. The starting materials and subsidiary colors found in the lot of R-3 evaluated were not present in large quantities except for 2,4,5-triiodofluorescein (4.4%) and are generally not suspected of being strong carcinogens. The data supporting a finding of carcinogenicity for the color additive are relatively weak. One could conclude that the presence of impurities or subsidiary colors may not significantly affect risk assessment calculations.

The lack of any information on the radiochemical purity of the color makes the penetrance study uninterpretable. The R-19 penetrance data were used, and this probably leads to an overestimate of exposure through the skin.

Stability studies of the compound "on the shelf" will be helpful for the clearer understanding of the toxic effects of R-3, especially when the phototoxic properties of the color are considered. This, and an adequate skin absorption study using live animals (e.g., pig) or people (using color labeled with non-toxic tracers such as the heavy, nonradioactive isotopes of carbon, nitrogen, etc.) would be helpful in estimating the risk.

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