June 16, 2014

Colleen Rogers
Center for Drug Evaluation and Research
Food and Drug Administration
Building 22, Room 5411
10903 New Hampshire Avenue
Silver Spring, MD 20993

Re: Proposed Rule: Proposed Amendment of the Tentative Final Monograph, Federal Register, Vol. 78, No. 242, Tuesday, December 17, 2013.

Docket identification (ID) number: FDA-1975-N-0012

Regulatory Information Number: 0910-AF69

Dear Ms. Rogers:

The American Cleaning Institute (ACI)[1] appreciates this opportunity to provide comments on the proposed rule to amend the 1994 tentative final monograph (the 1994 TFM) for over-the-counter (OTC) topical antiseptic drug products to establish conditions under which OTC consumer antiseptic products intended for use with water (referred to as consumer antiseptic washes) are generally recognized as safe and effective.

ACI has a specific interest in chloroxylenol within the proposed rule since our members produce antiseptic products containing chloroxylenol, as well as manufacture chloroxylenol and, as such, would be regulated under the FDA proposed rule. These products play a beneficial role in the supporting the health and hygiene of millions of people throughout the U.S. and worldwide. Chloroxylenol and chloroxylenol-containing products have a long track record of human and environmental safety which is supported by science-based, transparent risk analyses. They have been and are used safely and effectively in homes, hospitals, schools and workplaces.

ACI members are concerned that FDA did not appropriately assess the safety data that are available prior to proposing that additional data are necessary to support the use of chloroxylenol in consumer antiseptic washes. Chloroxylenol is a well-studied active ingredient for OTC antiseptic use for both safety and efficacy. However, it appears FDA had not considered the

[1] ACI is a trade association representing the $30 billion U.S. cleaning products industry. ACI members include the formulators of soaps, detergents, and general cleaning products used in household, commercial, industrial and institutional settings; companies that supply ingredients and finished packaging for these products; and oleochemical producers.

existence of relevant information beyond what is reported in the notice, including both published studies and unpublished studies, some of which were overlooked by FDA despite being part of the docket. Studies related to the safety and efficacy of chloroxylenol are described and cited in the Attachment to this letter ("FDA Consumer Antiseptics Rule: FDA Request for Data on Safety and Efficacy of Chloroxylenol," Exponent, 2014).

The following summarizes our response to the specific points raised in the proposed rule based on the findings presented in the Attachment:

1. The chloroxylenol database on absorption, distribution, metabolism and excretion (ADME) indicates that the information evaluated by FDA in the proposed rule fails to include relevant information from applicable studies that are available in the published literature and contained in studies that have been previously submitted to the agency.
2. Detailed evaluation of carcinogenicity following dermal exposure to chloroxylenol has been conducted and resulted in no finding of cancer. A similar carcinogenicity study following oral exposure is not available in the published literature or in unpublished studies. However, there is no clear need for an oral carcinogenicity study; the dermal carcinogenicity study provides a meaningful assessment of cancer risk from systemic exposures. Moreover, the relevant pathway of exposure for chloroxylenol is limited to dermal exposures, for which cancer risk is characterized.
3. The existing database of *in vitro* and *in vivo* studies indicates that chloroxylenol would not cause hormonal effects in humans at systemic concentrations resulting from topical exposure.
4. No relevant association between antibiotic resistance and chloroxylenol has been demonstrated.
5. Chloroxylenol has been shown to be effective at reducing the number of pathogenic bacteria in clinical environments.

In summary, the available toxicological information provides no evidence for data gaps or gives cause for concern under typical use conditions for chloroxylenol in consumer antiseptic wash products. Efficacy against pathogenic bacteria has been demonstrated. We believe that existing data on chloroxylenol is sufficient for demonstrating its safety and efficacy. Evaluation of the data in accordance with established principles, utilizing a weight of evidence approach, should lead FDA to conclude chloroxylenol deserves the official status of generally recognized as safe and effective.

FDA should also consider the safety assessments of chloroxylenol conducted by other authorities. For example, chloroxylenol has been reviewed and is permitted for use within the European Union (EU), as follows:

- Chloroxylenol is permitted for use in cosmetic products in accordance with EU Regulation EC/1223/2009, which contains a provision for chloroxylenol at a level of up to 0.5%. (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:en:PDF).

- Chloroxylenol is permitted for use in a number of topical pharmaceutical products, as licensed by the UK Medicines and Health Regulatory Agency (MHRA) (http://www.mhra.gov.uk/Safetyinformation/Medicinesinformation/SPCandPILs/index.htm?subsName=CHLOROXYLENOL&pageID=SecondLevel)

We ask the FDA to take reviews and assessments such as these into consideration.

As an industry body, we remain committed to the process of evaluating the safety and efficacy of chloroxylenol. In order to provide sufficient time to complete the studies that may turn out to be necessary to complete this process, a formal request is made that the FDA extend the time period allowed for submission of new data by a further 4 years, to allow 5 years in total to complete the work. An additional 4 years would allow time for (i) protocols to be agreed, (ii) contracts signed, (iii) studies to be performed, and (iv) final reports issued.

ACI also urges FDA to reconsider the new efficacy testing requirements presented in its proposed rule, which are unprecedented. Given the significance of the proposed change to the testing requirements for consumer antiseptics and the lack of precedent for this action, FDA should withdraw the proposed rule. It should be reissued as an Advance Notice of Proposed Rulemaking (ANPR) to give industry and other stakeholders an opportunity to engage with FDA on the generally recognized as effective (GRAE) testing requirements for the active ingredients and surrogate endpoint testing of final formulations.

FDA's efficacy requirements are unjustified by the risk-benefit analysis. Typically, reassessments of benefits and risks are prompted by a safety signal, such as the appearance of a particular sign, symptom, or symptom-complex. However, there has been no demonstration of a scientifically confirmed risk associated with the usage of consumer antiseptic products; there is only speculation around potential risks associated with endocrine disruption and antimicrobial resistance, without consideration of the full weight of evidence or a properly conducted risk assessment. This reliance on speculation to justify unparalleled testing requirements is unwarranted and is not justified on a scientific basis. Furthermore, the FDA does not appear to have considered the potential risks for having an increase in infection(s), including food-borne illness(es), among consumers by denying access to antibacterial product formulations. This is consistent with FDA's failure to make public their assessment of a safety risk in accordance with accepted transparent scientific principles recognized by the agency.

Further, FDA's proposed clinical trial requirements are unrealistic and infeasible. We believe that the testing of active ingredients for efficacy, rather than a formulation, is unnecessary and counter to the positions taken by FDA during the lifetime of this monograph as well as other monographs. To this end, we ask that FDA clearly differentiate between active ingredient and final formulation requirements, as well as consider simulation testing and surrogate endpoints which are more reasonable than testing for reduced infection rate.

We urge FDA to revise its proposed *in vitro* testing methods. ACI recommends that FDA require MIC/MLC testing of active ingredients on the ATCC reference strains described in the proposed rule to determine the spectrum of antibacterial activity. ACI urges the FDA to adopt American Society for Testing and Materials (ASTM) Method E2783 (Standard Test Method for Assessment of Antimicrobial Activity for Water Miscible Compounds Using a Time-Kill

Procedure) as the standard for conducting the Time-Kill testing for speed of antimicrobial effect for evaluation of formulated antiseptics. We request that FDA reconsider the performance criteria, which are more demanding than the performance abilities of approved healthcare antiseptic products and likely the unformulated active ingredients.

Recognizing that the use of standardized test methods is critical for regulatory testing and approval to assure consistency, FDA should adopt, as appropriate, established and accepted methodology to support the surrogate endpoint efficacy testing for finished antiseptic formulations, such as the following ASTM methods: E1174 - Standard Method for the Evaluation of Health Care Handwash Formulation, E2783 - Standard Test Method for Assessment of Antimicrobial Activity for Water Miscible Compounds Using a Time-Kill Procedure, and E2784 - Standard Test Method for Evaluation of the Effectiveness of Handwash Formulations Using the Paper Towel (Palmar) Method of Hand Contamination.

ACI can provide copies of the cited studies and reports upon request. We are willing to meet with you to review them in detail. Please contact me if you have any questions on these comments.

Sincerely,

Richard Sedlak
Executive Vice President, Technical & International Affairs

Exponent®

FDA Consumer Antiseptics Rule: FDA Request for Data on Safety and Efficacy of Chloroxylenol

Exponent®

FDA Consumer Antiseptics Rule: FDA Request for Data on Safety and Efficacy of Chloroxylenol

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June 12, 2014

Doc. no. 1402560.000 - 7266

Contents

Chloroxylenol: Review of Safety and Efficacy

At the request of the American Cleaning Institute (ACI), Exponent has reviewed the available information regarding the safety and efficacy of the antimicrobial chloroxylenol. This effort was conducted in response to a Proposed Rule and reopening of the U.S. Food and Drug Administration's (FDA's) administrative record on topical antimicrobial drug products for over-the-counter human use (Federal Register, Tuesday, December 17, 2013). The Proposed Rule addresses a variety of consumer antiseptic active ingredients. Exponent's effort, and the evaluation and comments presented in this document, focus specifically on information pertaining to the chemical chloroxylenol.

Chloroxylenol has a long history of safe use as an antimicrobial worldwide. In the U.S., a number of uses for chloroxylenol were tested in the 1950s, and there was a resurgence in interest in this antimicrobial since 1972, because many companies chose it to replace hexachlorophene in a variety of products, including surgical hand scrubs and hand-washing products and as preservatives in cosmetics and cutting oils (Bruch 1996). In December 2013, the FDA issued a Proposed Rule that reopened the administrative record on the *"Safety and Effectiveness of Consumer Antiseptics: Topical Antimicrobial Drug Products for Over-the-Counter Human Use"* ("Proposed Rule"). In the Proposed Rule, FDA states that "additional safety data are necessary to support the safety of antiseptic active ingredients," as well as information to support the effectiveness and potential for development of resistance.

In response to the Proposed Rule, Exponent staff evaluated the availability of information on chloroxylenol in the specific technical areas outlined by FDA:

- Efficacy
- Potential for the development of microbial resistance and cross-resistance to antibiotics
- Pharmacokinetic studies
- Absorption, distribution, metabolism, and excretion
- Effect of formulation on dermal absorption
- Dermal carcinogenicity
- Oral carcinogenicity
- DART studies of the effects on fertility and pre- and postnatal development
- Potential hormonal effects.

The overarching goal of this effort was to determine what information has already been identified by FDA to address these technical areas (as defined by the specific studies referenced

in the Proposed Rule), and to identify additional studies or other sources of information that FDA should include in any evaluation of the safety and efficacy of chloroxylenol.

A formal literature search was conducted to identify new literature. Because the *Proposed Rule* intends to amend the 1994 FDA tentative final monograph (TFM), records in this search were limited to materials published since 1994. Using relevant toxicology, chemistry, life sciences, and other scientific databases on the STN and ProQuest DIALOG search services, including but not limited to RTECS (Registry of Toxic Effects of Chemical Substances), Chemical Abstracts, Toxcenter, ToxFile, Toxicology Abstracts, TOXLINE, Medline, and Endocrinology Abstracts, a search was done using a variety of keywords and keyword combinations. The Chemical Abstracts Registry (CAS) number was included as a search term, in addition to the various synonyms for chloroxylenol identified in the CAS record, and these terms were paired with terms referring to the various toxic and carcinogenic effects of interest in this literature review. The records identified by this search were reviewed by the technical project team members for relevance in each specific technical area addressed.

Additionally, individualized literature searches were conducted by Exponent staff working within each technical area. These included focused searches of PubMed and other databases, as well as specific document retrieval based on information cited in other studies. The bibliography to support each of the technical evaluations is provided in each of the appendices to this report. The appendices also include information about cited studies in a tabular format.

Each of the technical areas bulleted above is discussed in a separate summary report, included herein as Appendices A–F. Each summary presents the information that FDA has listed as available to them, together with a discussion of additional information that should be considered by FDA. An overview of the findings for each technical area is summarized below, and a synthesis of the studies discussed in these technical appendices is provided in Table 1. This table lists all of the studies mentioned in the appendices, and delineates whether the FDA acknowledges the study, and which technical area(s) it addresses. The identified studies that are not included by FDA in the Proposed Rule include more recent publications, unpublished studies, and updated review articles. In some instances, Exponent's evaluation indicates that a number of studies have already been submitted to FDA, but the study is not included in the December 2013 Federal Register Notice for chloroxylenol. In these cases, FDA appears to have overlooked studies that are relevant and are already in the system and earmarked as providing data related to chloroxylenol. In other instances, studies may be new, or otherwise unknown to FDA.

Our review included studies that were specifically designed to address the safety and efficacy issues evaluated by FDA. In other instances, Exponent identified studies that evaluated relevant endpoints, even though those endpoints may not have been the specific focus of the study design. One example of this relates to evaluating the hormonal effects of chloroxylenol: some toxicity studies provide data that are directly applicable to evaluating the potential hormonal effects, although the study itself is designed to evaluate general toxicity and is not specifically focused on hormonal effects. Similarly, there are instances in which studies on diverse product types provide information that is potentially relevant to FDA's re-evaluation, such as studies on the absorption of chloroxylenol from metalworking fluid that evaluate the effect of mixtures on absorption.

We are pleased to provide this information for consideration of the safety and effectiveness of this well-studied antimicrobial, which has a long history of safe use in the United States and worldwide, under a wide variety of applications.

Overview and Summary of Technical Reviews

Below is a summary of the conclusions drawn in each of the attached appendices.

Appendix A: Chloroxylenol Antimicrobial Efficacy & Resistance

A literature search did not reveal additional studies that evaluated chloroxylenol-containing hand soap efficacy in preventing human clinical disease in household (consumer) environments. However, a number of publications describe clinical disease-reduction efficacy in other use-environments, including food handling and hospital/clinical applications. Despite the long worldwide history of chloroxylenol use in household environments, the literature search did not reveal any evidence that exposures resulted in the emergence of resistant bacterial isolates. Several studies evaluated exposures in household, clinical, and other application environments, and there was no indication of either cross-antimicrobial or antibiotic resistance induction at use-dilutions of chloroxylenol. The persistence of chloroxylenol in the environment is limited, further reducing the risk of long-term, low-level exposures that could lead to development of resistant isolates.

Appendix B: Assessment of the Developmental and Reproductive Toxicity (DART) Database for Chloroxylenol

Three rat developmental toxicity studies of chloroxylenol are available. These studies establish developmental no-observed-effect levels (NOELs) of 300 and 1,000 mg/kg/day. No rabbit developmental toxicity studies were identified. However, because the one rat teratology study (Siglin 1991) has been characterized by FDA as "adequately characterizes chloroxylenol's potential effects on embryo and fetal development," FDA should not require a rabbit study in this circumstance.

In addition to the three rat developmental toxicity studies, a multi-generation rat reproduction study (Harr 1978) is also available and has been summarized in a review by Guess and Bruch (1986). Although the adequacy of this study cannot be determined based on the limited information provided in the review, this study should be considered by FDA in order to fully characterize chloroxylenol's potential to cause development and reproduction toxicity.

Appendix C: Assessment of the Hormonal Effects Database for Chloroxylenol

Ten *in silico/in vitro* studies that assess chloroxylenol's ability to interact with the estrogen receptor (nine studies) and the androgen receptor (one study) are available in the open literature. In all cases, chloroxylenol was found to have moderately weak receptor activity. Consequently,

it is likely that relatively high concentrations of the compound would be required to induce a relevant effect in an intact animal.

In addition to the ten *in silico/in vitro* studies mentioned above, numerous *in vivo* studies conducted to fulfill regulatory requirements are available that assess chloroxylenol's activity in the intact animal. Overall, these *in vivo* studies show that chloroxylenol has no effect on hormonally sensitive endpoints, including the weights and histopathology of hormonally sensitive tissues and the expression of hormonally sensitive parameters in DART studies. Doses administered in at least some of these studies reached the generally accepted limit dose of 1,000 mg/kg/day.

Taken together, the available data indicate that, although chloroxylenol may demonstrate weak receptor activity in *in vitro* test systems, results from *in vivo* studies demonstrate that the hormonal activity of this chemical is too weak to interact at the estrogen or androgen receptor to induce hormonal effects in the intact animal.

Appendix D: Assessment of the Mutagenic and Carcinogenic Effects for Chloroxylenol

In the Federal Register Notice, FDA identified only one 13-week repeated-dose dermal toxicity study in mice. Literature searches conducted by Exponent identified a dermal carcinogenicity study, additional repeated-dose dermal toxicity studies, oral repeated-dose toxicity studies, and genotoxicity studies conducted with chloroxylenol, all of which should be considered by the FDA in their evaluation of chloroxylenol.

In an 18-month dermal carcinogenicity study in mice, no evidence of carcinogenicity was found. No oral carcinogenicity studies were located in the literature for chloroxylenol. However, based on a weight-of-evidence-based approach, the potential for chloroxylenol to result in oral carcinogenicity is low: oral repeated-dose toxicity studies provided no indication that cancer effects are anticipated, and studies in animals fail to demonstrate genotoxicity. Additionally, because chloroxylenol has been experimentally demonstrated to be absorbed through the skin in rats, the dermal carcinogenicity studies in rodents do indicate that there is no carcinogenic response following systemic exposure to chloroxylenol. Moreover, oral exposure is not anticipated for products containing chloroxylenol; therefore, the dermal carcinogenicity study that has been performed provides the relevant information for a safety evaluation of human uses of chloroxylenol.

Appendix E: Assessment of the Pharmacokinetics (ADME) Database for Chloroxylenol

Chloroxylenol has been used safely in a variety of dermally applied consumer products; therefore, few animal or human studies have been conducted to evaluate the ADME of chloroxylenol in humans or animals. FDA has evaluated some of the available studies, but concluded that more studies are necessary. A review of the literature has identified additional studies that were not discussed or evaluated by FDA, and that could enhance the understanding of the ADME of chloroxylenol. Together, the available ADME studies indicate that

chloroxylenol can be absorbed through the skin, but it is metabolized and excreted rapidly, and blood levels cannot be detected unless high doses are administered.

Appendix F: Assessment of the Percutaneous Absorption of Chloroxylenol, and Effects of Formulation

Because of the importance of the dermal exposure pathway for human exposure to chloroxylenol in consumer products, a specific evaluation focused on dermal absorption was conducted. This review indicates that studies on the dermal absorption of chloroxylenol have been conducted on several species of research animals, and in studies using human volunteers. Taken together, the available data on the dermal absorption of chloroxylenol indicate that it can be absorbed following dermal application of high doses, and that absorption is enhanced by damaged (abraded) skin, high concentrations, extended dosing periods, and occlusive covering.

Many of the studies conducted on high concentrations, high loadings, and/or for long periods of exposure provide results that are not relevant to understanding the potential for human exposure from actual uses of products containing chloroxylenol, and these considerations should be evaluated by FDA in their evaluation. Newer studies that were not included in FDA's evaluation, some of which were performed to understand percutaneous absorption of chloroxylenol from occupational settings, provide more detailed evaluations, including rates of absorption at lower dermal loading rates than those used in older studies. Results from these studies may be more useful for understanding dermal absorption of chloroxylenol from consumer products than are data generated from studies based on extreme exposure conditions.

Table 1. Summary of studies supporting technical evaluation of chloroxylenol

Author	Date	Title	Cited in Proposed Rule	Relevant to Efficacy and Resistance	Relevant to DART	to Hormonal Effects	Relevant to Carcinogenicity	Relevant to ADME	Relevant to Dermal Abs
Gibson, L.L., J.B. Rose, C.N. Haas, C.P. Gerba, and P.A. Rusin	2002	Quantitative assessment of risk reduction from hand washing with antibacterial soaps. J Appl Microbiol 92:136S–143S.		√					
Goddard, P.A. and K.A. McCue	2001	Phenolic compounds. Disinfection, sterilization, and preservation, 5th Ed. SS Block, ed. Lippincott Williams & Wilkins, Malvern, PA, pp. 255–281.		√					
Gudipati, R.M. and S.A. Stavchansky	1995	Percutaneous absorption of parachlorometaxylenol. Intl J Pharmaceutics 118:41–45.						√	√
Guess, W.L. and M.K. Bruch	1986	A review of available toxicity data on the topical antimicrobial, Chloroxylenol. J. Toxicol.-Cut. & Ocular Toxicol. 5(4)233-262.		√	√	√	√	√	√
Haas, C.N., J.R. Marie, J.B. Rose, and C.P. Gerba	2005	Assessment of benefits from use of antimicrobial hand products: Reduction in risk from handling ground beef. Intl J Hyg Environ Health 208(6):461–466.		√					
Harr, J.R.	1978	Pennwalt Corporation.			√	√	√		
Havler ME, Rance MJ. 1977.	1977	The metabolism of p-chloro-m-xylenol (PCMX) in Sprague Dawley and Gunn Wistar rats. Reckitt & Colman, in FDA Docket No. 1975N–0183H .	√					√	
Havler, M.E., B.J. Jordan, S. Malam, and M.J. Rance	1974	Metabolism studies of PCMX. Report No. 5369/2. Reckitt and Colman Co. Submitted to FDA Docket 175N-0183.							√
Houtman, C.J., A.M. Van Oostveen, A. Brouwer, M.H. Lamoree, and J. Legler	2004	Identification of estrogenic compounds in fish bile using bioassay-directed fractionation. Environ Sci Technol 38:6415–6423.				√			
Hunter, B., J.L. Bridges, A.J. Newman	1973	Dettol RBA 666 preliminary assessment of toxicity to rats, oral administration for four weeks. Huntingdon Research Centre. FDA Docket No. 75N-0183 (as cited in Guess and Bruch 1986).				√	√		
Hunter, B., J.L. Bridges, R. Heywood, and A.E. Street	1973	RBA 666 toxicity to rats in oral administration for 13 weeks. Huntington Research Centre. RKT46/73744. December 12. FDA Docket No. 75N-0183				√	√		
Hutton, D.B.	1998	Use of household disinfectants to suppress Pratylenchus coffeae and dry rot of yellow yam (Dioscorea cayenensis). Trop Agric 75(1-2):49–52.		√					
International Conference on Harmonisation (ICH)	2000	ICH harmonised tripartite guideline: Safety pharmacology studies for human pharmaceuticals S7A. Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S7A/Step4/S7A_Guideline.pdf	√					√	
International Conference on Harmonisation (ICH)	1994	Guideline for industry: Detection of toxicity to reproduction for medicinal products. ICH-S5A. September. Available at www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm065007.htm.			√				
International Conference on Harmonisation (ICH)	1996	Guideline for industry: The need for long-term rodent carcinogenicity studies of pharmaceuticals. ICH-S1A. March. Available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM074911.pdf					√		
International Conference on Harmonisation (ICH)	1997	Guideline for industry: A standard battery for genotoxicity testing of pharmaceuticals. ICH-S2B. November. Available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM074929.pdf					√		
International Conference on Harmonisation (ICH)	1997	Guideline for industry: S1B testing for carcinogenciity of pharmaceuticals. ICH-S1B. July. Available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM074916.pdf					√		
Ivett, J.	1989	Mutagenicity test on chloroxylenol in the in vivo mouse micronucleus assay: final report: Project ID: HLA Study No. 10555-0-455. Unpublished study prepared by Hazleton Laboratories America, Inc. 21 p. MRID 41085301					√		
Jain, P.K. and P.C. Gangwar	1972	Effects of storage and antibiotic treatments on developmental malformations in chickens. Indian J. Exp. Biol., 10:319-321.			√				
Jordan, B.J. et al.	1973	Human volunteer studies on Dettol bathing product, FDA Docket 1975n-0183H	√					√	

Table 1. (cont.)

Author	Date	Title	Cited in Proposed Rule	Relevant to Efficacy and Resistance	Relevant to DART	to Hormonal Effects	Relevant to Carcinogenicity	Relevant to ADME	Relevant to Dermal Abs
Jordan, B.J., J.D. Nichols, M.J. Rance	1973	Dettol Bathing Product - Preliminary Volunteer Study, FDA Docket 1975n-0183H	√					√	
Klarenbeek, A.	1954	An investigation into the viricidal action of Teepol, sodium hydroxide, propylene glycol and Dettol. J Hyg 52(4):529–533.		√					
Klopman G., and S.K. Chakravarti	2003	Screening of high production volume chemicals for estrogen receptor binding activity (II) by the MultiCASE expert system. Chemosphere 51:461–468.				√			
Lambert, R.J.W.	2004	Comparative analysis of antibiotic and antimicrobial biocide susceptibility data in clinical isolates of methicillin-sensitive *Staphylococcus aureus* , methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* between 1989 and 2000. J. Applied Microbiology 97:699-711.	√	√					
Lear, J.C., J.Y. Maillard, P.W. Dettmar, P.A. Goddard, A.D. Russell	2002	Chloroxylenol- and triclosan-tolerant bacteria from industrial sources. J Indust Microbiol Biotechnol 29(5):238–242.	√	√					
Lear, J.C., J.Y. Maillard, P.W. Dettmar, P.A. Goddard, A.D. Russell	2006	Chloroxylenol- and triclosan-tolerant bacteria from industrial sources — Susceptibility to antibiotics and other biocides. Int Biodeterior Biodegrad	√	√					
Maes, P.S., J. Li, E. Verbeeck, J. Keyaerts, J. Clement, and M. Van Ranst	2007	Evaluation of the efficacy of disinfectants against Puumala hantavirus by real-time Rt-PCR. J Virol Methods 141(1):111–115.		√					
Maillard, J.Y. and S.P. Denyer	2009	Emerging bacterial resistance following biocide exposure: Should we be concerned? Chim Oggi-Chem Today 27(3):26–28.		√					
Mansouri, M.D. and R.O. Darouiche	2008	In-vitro activity and in-vivo efficacy of catheters impregnated with chloroxylenol and thymol against uropathogens. Clin Microbiol Infect 14(2):190–192.		√					
Marshall, B.M., and L.M. McMurry	2005	Biocides and resistance. Frontiers in antimicrobial resistance: A tribute to Stuart B. Levy. DG White, MN Alekshun, PF McDermott, Eds. ASM Press, Washington, DC, pp. 174–190.		√					
Marshall, B.M., E. Robleto, T. Dumont, and S.B. Levy	2012	The frequency of antibiotic-resistant bacteria in homes differing in their use of surface antibacterial agents. Curr Microbiol 65(4):407–415.		√					
Mastri, C.W., and M.L. Kepling	1973	28-day subacute dermal toxicity study with AT0029D in Albino rabbits. Unpublished Study No. A2814. Industrial Bio-Test Laboratories, Inc., Northbrook,					√		
May, K.	1989	Nipacide MX (parachlorometaxylenol): Assessment of mutagenic potential in histidine auxotrophs of salmonella typhmurium: Lab Project Number 89/0690. NIPA/1989/8. Unpublished study prepared by Life Science Research Ltd. 32 p. MRID 41310301					√		
McDonnell, G. and A.D. Russell	1999	Antiseptics and disinfectants: Activity, action, and resistance. Clin Microbiol Rev 12(1):147–179.		√					
McMurry, L.M., M. Oethinger, and S.B. Levy	1998	Triclosan targets lipid synthesis. Nature 394:531–532.		√					
Messager, S., P.A. Goddard, P.W. Dettmar, and J.Y. Maillard	2001	Determination of the antibacterial efficacy of several antiseptics tested on skin by an 'ex-vivo' test. J Med Microbiol 50(3):284–292.		√					
Messager, S., P.A. Goddard, P.W. Dettmar, and J.Y. Maillard	2004	Comparison of two in vivo and two ex vivo tests to assess the antibacterial activity of several antiseptics. J Hosp Infect 58(2):115–121.		√					
Miller, D., B.B. Wheals, N. Beresford and J.P. Sumpter	2001	Estrogenic activity of phenolic additives determined by an *In Vitro* yeast bioassay. Environ. Health Perspect. 109:133-138.				√			
Mombelli, E.	2011	Evaluation of the OECD (Q)SAR application toolbox for the profiling of estrogen receptor binding affinities. SAR QSAR Environ Res 23:37–57.				√			
Momma, J., K. Takada, Y. Aida, H. Yoshimoto, K. Naito, Y. Suzuki, Y. Nakaji, Y. Kurokawa, and M. Tobe	1988	Combined long-term toxicity and carcinogenicity test of p-chloro-m-xylenol (PCMX) applied to female mouse skin. Bulletin of National Institute of Hygenic Sciences, 106(63)39-47.				√	√		
Montville, R. and D.W. Schaffner	2011	A meta-analysis of the published literature on the effectiveness of antimicrobial soaps. J Food Prot 74(11):1875–1882.		√					
Morris, T.D.	2002	A 13-week dermal toxicity study of PCMX in mice - Clariant. Study No. WIL-304003. WIL Research Laboratories, Inc., Ashland, OH. July 2.	√			√	√		
Nakama, A., K. Funasaka, and M. Shimizu	2007	Evaluation of estrogenic activity of organic biocides using ER-binding and YES assay. Food Chem Toxicol 45:1558-1564.				√			

Table 1. (cont.)

Author	Date	Title	Cited in Proposed Rule	Relevant to Efficacy and Resistance	Relevant to DART	to Hormonal Effects	Relevant to Carcinogenicity	Relevant to ADME	Relevant to Dermal Abs
Nakano, S., Y. Nagao, T. Kobyashi, M. Tanaka, S. Hirano, Y. Nobuhara, and T. Yamada	2002	Problems with methods used to screen estrogenic chemicals by yeast two-hybrid assays. J. Health Science 48(1)83-88.				√			
Nishihara, T., J-i. Nishikawa, t. Kanayama, F. Dakeyama, K. Saito, M. Imagawa, S. Takatori, Y. Kitagawa, S. Hori, and H. Utsumi	2000	Estrogenic activities of 517 chemicals by yeast two-hybrid assay. J. Health Science 46(4)282-298.				√			
Noda, T., S. Morita, A. Yamada, and S. Ohgaki	1983	Safety evaluation of chemicals for use in household products (IV) teratological studies on p-Chloro-m-xylenol in rats. Annual report of Osaka City Institute of Public Health and Environmental Sciences, pp. 100-105.			√	√			
O'Connor, D.O., and J.R. Rubino	1991	Phenolic compounds. Disinfection, sterilization, and preservation, 4th Ed. SS Block, Ed. Lea and Febiger, Philadelphia, PA. pp. 204–224.		√					
Ogbulie, J.N., I.E. Adieze, and N.C. Nwankwo	2008	Susceptibility pattern of some clinical bacterial isolates to selected antibiotics and disinfectants. Pol J Microbiol 57(3):199–204.		√					
Payne, D.N., J.R. Babb, and C.R. Bradley	1999	An evaluation of the suitability of the european suspension test to reflect in vitro activity of antiseptics against clinically significant organisms. Lett Appl Microbiol 28(1):7–12.		√					
Payne, D.N., S.A.W. Gibson, and R. Lewis	1998	Antiseptics: A forgotten weapon in the control of antibiotic resistant bacteria in hospital and community settings? J R Soc Health 118(1):18–22.		√					
Rhoades, J.K., M. Gialagkolidou, O. Gogou, N. Mavridou, C. Blatsiotis, C. Ritzoulis, and E. Likotrafiti	2013	Oregano essential oil as an antimicrobial additive to detergent for hand washing and food contact surface cleaning. J Appl Microbiol 115(4):987–994.		√					
Roberts, M.S., R.A. Anderson, and J. Swarbrick	1977	Permeability of human epidermis to phenolic compounds. J Pharm Pharmac 29:677–683.						√	√
Rostkowski, P., J. Horwood, J. Shears, A. Lange, F. Oladapo, H.T. Besselink, C.R. Tyler, and E.M. Hill	2011	Bioassay-directed identification of novel antiandrogenic compounds in bile of fish exposed to wastewater effluents. Environ. Sci. & Technol. 45:10660-10667.				√			
Russell, A.D.	2003	Biocide use and antibiotic resistance: The relevance of laboratory findings to clinical and environmental situations. Lancet Infect Dis 3(12):794–803.		√					
Russell, A.D.	2004	Whither triclosan? J Antimicrob Chemother 53(5):693–695.		√					
SCENIHR	2009	Assessment of the antibiotic resistance effects of biocides.		√					
Schaffner, D.W., J.P. Bowman, D.J. English, G.E. Fischler, J.L. Fuls, J.F. Krowka, and F.H. Kruszewski	2014	Quantitative microbial risk assessment of antibacterial (h)and hygiene products on risk of shigellosis. J Food Prot 77(4):574–582.		√					
Sheena, A.Z., and M.E. Stiles	1983	Efficacy of germicidal hand wash agents against transient bacteria inoculated onto hands. J Food Prot 46(8):722–727.		√					
Siglin, J.C.	1991	4 Chloro 3,5 xylenol (PCMX) teratology study in rats. Springborn Laboratories, Inc. Nipa-1991-4. July 10, 1991.	√		√	√			
Stavchansky, S.	1985	Report to Dexide, Inc. Submitted to FDA Docket 175N-0183.							√
Sved, D.W. et al.	2000	A dermal absorption study with [14c]-labeled PCMX in mice. In FDA Docket No. 1975N–0183H.	√					√	
Terasaka, S., A. Inoue, M. Tanji, and R. Kiyama	2006	Expression profiling of estrogen-responsive genes in breast cancer cells treated with alkylphenols, chlorinated phenols, parabens, or bis- and benzoylphenols for evaluation of estrogenic activity. Toxicol. Letters 163:130-141.				√			
Thomas, R.E. and A.T. Kotchevar	2010	Comparative in vitro metabolism of chloroxylenol, chlorophene, and triclosan with rat, mouse, and human hepatic microsomes. Toxicological & Environmental Chemistry 92(9)1735-1747.						√	
ToxNet	2014	Chloroxylenol, CASRN: 88-04-0		√					

Table 1. (cont.)

Author	Date	Title	Cited in Proposed Rule	Relevant to Efficacy and Resistance	Relevant to DART	to Hormonal Effects	Relevant to Carcinogenicity	Relevant to ADME	Relevant to Dermal Abs
U.S. EPA	1994	R.E.D. Facts pesticide reregistration - Chloroxylenol. EPA-738-F-94-028.		√					
U.S. EPA	1994	Reregistration eligibility decision RED). Chloroxylenol. EPA 738-R-94-032. September 1994. U.S. Environmental Protection Agency.		√	√	√		√	√
U.S. EPA	2009	Chloroxylenol summary document: Registration review preliminary work plan. EPA-HQ-OPP-2009-0010. March. U.S. Environmental Protection Agency.		√	√	√		√	√
U.S. EPA	2009	Summary of human health effects data for the chloroxylenol registration review decision document. EPA-HQ-OPP-2009-0010-001. March 4. U.S. Environmental Protection Agency.			√	√	√		√
U.S. FDA	1987	Guideline for the format and content of the nonclinical pharmacology/toxicology section of an application. U.S. Food and Drug Administration. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM079234.pdf.	√					√	
U.S. FDA	2005	Guidance for industry. Acne vulgaris: Developing drugs for treatment. U.S. Food and Drug Administration. Available at: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM071292.pdf	√					√	
U.S. FDA	2013	Safety and effectiveness of consumer antiseptics; topical antimicrobial drug products for over-the-counter human use; proposed amendment of the tentative final monograph; reopening of Administrative Record. 21 CFR Parts 310 and 333. U.S. Food and Drug Administration. Fed Reg 78:76444–76478.	√	√	√	√		√	
U.S. FDA	2013	Guidance for industry. Endocrine disruption potential of drugs: Nonclinical evaluation. Draft guidance. September.				√			
Vijay, V., E.M. White, M.D. Kaminski Fr., J.E. Riviere, and R.E. Baynes	2009	Dermal permeation of biocides and aromatic chemicals in three generic formulations of metalworking fluids. J Toxicol Environ Health, Part A: Current		√				√	√
Zondek, B.	1942	The excretion of halogenated phenols and their use in the treatment of urogenital infections. J. Urol. 48:747–758.						√	√
Zondek, B., and B. Shapiro	1943	Fate of halogenated phenols in the organism. Biomed J 37:592–595.						√	
Zondek, B., and M. Finkelstein	1946	Blood concentration of p-chloroxylenol in man following parenteral percutaneous and rectal application. Proc Soc Exp Biol Med 61:200–202.						√	√

Appendix A

Efficacy and Resistance of Chloroxylenol

Appendix A: Chloroxylenol Antimicrobial Efficacy & Resistance

Summary of Findings

The literature search that is the subject of this appendix did not reveal additional studies that evaluated chloroxylenol-containing hand soap efficacy in preventing human clinical disease in household (consumer) environments. However, a number of publications describe clinical disease-reduction efficacy in other use-environments, including food handling and hospital/clinical applications. Despite the long worldwide history of chloroxylenol use in household environments, the literature search did not reveal any evidence that exposures resulted in the emergence of resistant bacterial isolates. Several studies evaluated exposures in household, clinical, and other application environments, and there was no indication of either cross-antimicrobial or antibiotic resistance induction at use-dilutions of chloroxylenol. The persistence of chloroxylenol in the environment is limited, further reducing the risk of long-term, low-level exposures that could lead to development of resistant isolates.

Background

Chloroxylenol has been used in the U.S. since the early 1950s, and was first registered for use as a fungicide in 1959 (U.S. EPA 2009). Chloroxylenol is the active ingredient in Dettol, which is a household disinfectant solution that is widely used in the U.K. and in a number of commonwealth countries. In the U.S., chloroxylenol is used as an antimicrobial in liquid hand soaps, household and food processing disinfectants, antiseptic surgical scrubs, and as a preservative in a variety of consumer and industrial liquid formulations. Chloroxylenol exhibits broad-spectrum *in vitro* and *in vivo* antimicrobial activity against a number of Gram+ and Gram– bacteria, fungi, and viruses, although the specific activity is formulation dependent. The mode of chloroxylenol antimicrobial action, as with other phenolics, involves damage to the cell (cytoplasmic) membrane of bacteria and fungi; the mode-of-action against viruses (enveloped and non-enveloped) is not well understood (Goddard and McCue 2001).

In the Proposed Rule (CFR 2013), FDA acknowledges that some data related to chloroxylenol antimicrobial efficacy and antimicrobial resistance development in liquid hand soaps are available, but conclude that the available data are inadequate. In response to the Proposed Rule, a literature search was performed, focusing on antimicrobial efficacy and resistance issues associated with chloroxylenol and related phenolic compounds.

The current Proposed Rule addresses the use of antimicrobials in hand washes for consumer use, but does exclude those antimicrobial-containing hand soaps and antiseptics used in health-care institutions. There are a significant number of peer-reviewed publications that support the use of chloroxylenol in hand soaps used in these environments, as well as in industrial, food services, and food production environments. The goal of this evaluation is to identify additional

studies (i.e., in addition to the information cited by FDA in the propose rule) that that would be appropriate for FDA to consider in their evaluation of chloroxylenol for antimicrobial efficacy and resistance development. Table A1 provides a representative survey of the literature related to chloroxylenol efficacy and resistance issues.

Table A1. Representative peer-reviewed publication survey*

Criterion	Demonstrated	Not Demonstrated	Marginal or unclear (insufficient data)	Consensus of Reviewed Publications
Mode-of-action	(O'Connor and Rubino 1991; McDonnell and Russell 1999; Goddard and McCue 2001)	not applicable	not applicable	Mode of action thought be similar to that of other phenolics; namely; multiple, non-specific sites of cell membrane denaturation
Antimicrobial efficacy, consumer	Sheena and Stiles, (1983); Haas et al. (2005); Rhoades et al. (2013)		Montville and Schaffer (2011)	A statistically significant, albeit marginal, improvement in microbial killing in liquid handsoap. Insufficient data concerning impact on infection rates associated with consumer use
Antimicrobial efficacy, clinical**	(Klarenbeek 1954; Payne et al. 1998; Payne et al. 1999; Messager et al. 2004; Butcher and Ulaeto 2005; Maes et al. 2007; Mansouri and Darouiche 2008; Ogbulie et al. 2008***; Payne et al. 1998***; Payne et al. 1999***)		Messager et al. (2001); El Mahmood and Doughari (2008)	Antimicrobial efficacy against a broad spectrum of Gram– and Gram+ bacteria is generally recognized, along with viruses. Clear evidence that handwashing with antimicrobial soaps reduces incidence of nosocomial infections.
Antimicrobial efficacy, food processing operations	Edmonds et al. (2012); Hutton (1998)			Effective in reducing pathogen loading associated with food processing operations. Effective for some crop treatment applications.
Antimicrobial resistance, consumer		**Lear et al. (2002); Lear et al. (2006)**	Maillard and Denyer (2009)	Insufficient data concerning the impact on antimicrobial resistance and/or cross-resistance in a consumer setting. No evidence of resistance development in industrial/ environmental exposures.
Antimicrobial resistance, clinical	Lambert (2004)****	Darouiche et al. (1998); **Lambert (2004);** Messager et al. (2004)		There is little evidence that chloroxylenol use by consumers or health-care institutions has increased resistant isolates or cross resistance to antimicrobials or antibiotics in clinical settings.

Note: chloroxylenol efficacy and antimicrobial resistance associated with liquid soap formulations. Studies listed by topic, with a summary of weight of evidence supported by the literature on each topic. Bold-faced entries are those also cited by the FDA in the Proposed Rule, cited above.

*May 5-22, 2014

**May also include studies of non-liquid soap formulations

***This was an *in vitro* study

****Gentamicin resistance (only) increased

Antimicrobial Efficacy

Chloroxylenol has a long history of use, particularly as a surface-disinfecting active ingredient in Dettol. A number of studies show *in vitro* effects of chloroxylenol on environmental isolates, including a number of putative pathogens in disinfectant solutions. There appear to be limited data (i.e., there is a "data gap") examining the impact that incorporation of chloroxylenol in consumer hand soaps has in reducing the risk of clinical disease. However, a relatively recent quantitative microbial risk assessment study (Schaffner et al. 2014) suggested that the addition of some antimicrobials (chloroxylenol was not included in this study) to liquid hand soaps used in food-handling applications significantly reduces the risk of clinical disease.

Bloomfield and Scott (2013) suggest that there may be instances where incorporation of antimicrobials into liquid hand soaps, etc., may be warranted in the household (consumer) environment:

- "Hygiene failure carries a risk of serious consequences (eg, handling raw contaminated food in the kitchen),
- surface rinsing is not possible (eg, tap handles, large surfaces),
- lack of access to a sink with soap and running water,
- microbes are strongly attached to the surface (eg, cloths), and
- infectious source (people, domestic animals) or at-risk groups are present in the home."

In addition, Gibson et al. (2002) concluded that addition of antimicrobial agents to hand soaps may result in "…slightly greater reduction of bacteria and subsequent reduced probability of disease."

As with other antiseptic agents, chloroxylenol has been shown to be effective at reducing numbers of pathogenic bacteria in clinical environments (Messager et al. 2001, 2004). Similarly, viral inactivation by chloroxylenol antiseptics has been demonstrated (Butcher and Ulaeto 2005; Maes et al. 2007). As was noted above, no published studies were identified that evaluated the efficacy of chloroxylenol-containing products in reducing clinical disease in household environments. However, there may be some applications in the consumer environment—e.g., handwashing following diaper changing—where the demonstrated clinical efficacy of chloroxylenol may support its incorporation into liquid hand soaps.

Antimicrobial Resistance and Cross-Resistance

The mode of action for antimicrobial phenolic compounds such as chloroxylenol has been studied for more than 100 years. While significant variations in activity levels exist as a function of the specific agent (and formulation), all phenolic compounds appear to interact with the cell membranes of bacteria and fungi. The specific interactions between chloroxylenol (as with many other phenolics and other antimicrobials) and the cell membrane are not well

understood. However, cell membrane constituents are disrupted with exposure to typical use-dilutions of phenolic compounds, including chloroxylenol (McDonnell and Russell 1999; Goddard and McCue 2001). At higher concentrations, there is also evidence that bacterial cell walls are disrupted, leading to cell death. It is likely that multiple cytoplasmic membrane targets (such as enzymes involved in transport functions) are affected by phenolic compounds such as chloroxylenol. While McMurry et al. (1998) have described a specific target enzyme associated with triclosan activity, there has been no evidence of in-use antimicrobial resistance, cross-resistance, or antibiotic resistance among environmental bacterial isolates associated with in-use concentrations of triclosan (Russell 2004) or other phenolic compounds.

A limited number of antimicrobial/antibiotic resistance studies involving chloroxylenol have been performed to date. The Lear et al studies (Lear et al. 2002, 2006) examined chloroxylenol in industrial/consumer settings, and did not find any evidence of either antimicrobial resistance/cross resistance development or of antibiotic resistance among environmental bacterial isolates. Lambert (2004) did not find any correlation between use of chloroxylenol (or most other antimicrobials) and antibiotic resistance among clinical strains of Staphylococcus aureus (MSSA and MRSA) or Pseudomonas aeruginosa. In general, there is no evidence that the use of household disinfectant cleaning agents has led to either disinfectant resistance/cross-resistance or antibiotic resistance. Marshall et al. (2012) concluded in their studies of this issue, "These findings contrast with those from in vitro laboratory exposures to biocides in which a variety of species has demonstrated elevated tolerance to biocides with concurrent cross-resistance to one or more antibiotics (Marshall and McMurry 2005). However, our findings concur with other literature reports on in situ exposures and comparisons. A study of 993 selected kitchen and bathroom isolates from 30 user and 30 non-user households found no cross-resistance between antibiotics and biocides (Cole et al. 2003)."

Despite a long history of chloroxylenol use in a variety of consumer and industrial product formulations, reports of enhanced antimicrobial or antibiotic resistance were not found in the peer-reviewed literature. The absence of such reports is not surprising: as was noted above, phenolic compounds have multiple target sites of action on bacteria, fungi, and viruses. While tolerance to low levels of antimicrobials—including substituted phenolics—in the environment has been reported, there is little evidence that resistance occurs at in-use concentrations (such as those used in liquid hand soaps), nor that antibiotic resistance is enhanced (Lear et al. 2002, 2006; Russell 2003).

Information regarding environmental persistence can be relevant to understanding the potential for the development of antibiotic resistance. There are limited studies of the fate on chloroxylenol in the environment. Based on physical and chemical properties, chloroxylenol released to the environment will partition between the atmosphere, water and soils. It has low volatility and low water solubility (EPA 1994), but will readily sorb to soils and sediments (Toxnet 2014). Chloroxylenol will not undergo hydrolysis, nor does it undergo direct photolysis in the presence of ultraviolet light (Toxnet 2014; Song 2009). However, chloroxylenol does breakdown in the presence of hydroxyl radicals in the atmosphere with a half-life of 6 hours (Toxnet 2014).

Chloroxylenol is rapidly degraded during wastewater treatment, particularly during biological treatment. Early reports suggested low levels of degradation were achieved during wastewater

treatment (Toxnet 2014). However, more recent studies have found chloroxylenol to be readily degradable in wastewater treatment plants (WWTPs) with biological treatment (either trickling filters or activated sludge), achieving greater than 80% degradation within five hours (Oppenheimer 2007; Kasprzyk-Hordern 2009). Chloroxylenol degradation studies using the fungus Aspergillus niger have also found rapid rates of biodegradation, with 100% removal within 6 days (Ghanem 2013). A single study investigating chloroxylenol concentrations in rivers found approximately 40% reduction in the concentration of chloroxylenol as it was transported 27 kilometers downstream of a WWTP discharge (Kasprzyk-Hordern 2008). This reduction in concentration appears to be due mainly to degradation. The decrease in chloroxylenol concentrations was similar and/or greater than that observed for other readily degradable and structurally similar chemicals (e.g. methylparaben) included in this study (Kasprzyk-Hordern 2008; Kasprzyk-Hordern 2009; Oppenheimer 2007). Taken together, this information indicates that chloroxylenol is readily degraded in the environment.

The literature teaches that resistance has not been demonstrated for chloroxylenol, despite many decades of use worldwide. Additionally, a direct study of rivers indicates that the persistence of chloroxylenol in the environment is limited, which further reduces the risk of non-target microbial exposure and the associated risk of resistance development.

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Literature Search

Major Search Terms/Sources (Web of Science, 5/5-5/22/14 by M.W. Mittelman)

- Larson, E. (2003) cited by
- Larson, E. and pcmx or chloroxylenol, topic
- Lear et al. (2002) cited by
- Lear et al. (2006) cited by
- Russell (2003) cited by (and including chloroxylenol or pcmx)
- chloroxylenol or pcmx and (efficacy or effectiveness)/title; topic
- chloroxylenol or pcmx and (detergent*), topic
- chloroxylenol or pcmx and (clean*), topic
- Dettol, topic
- 78 FR-242, December 17, 2013 references

The literature search was performed to identify peer-reviewed publications that addressed the putative data gaps noted by the FDA in their Proposed Rule. The literature search was expanded to encompass related uses of chloroxylenol in detergent (cleaning) formulations, healthcare washes and antiseptics, and in the food services industry. The purpose of this expanded search was to identify studies that described chloroxylenol efficacy and/or emergence of resistance in the use-environment.

The results of this literature search failed to reveal studies that evaluated chloroxylenol-containing hand soap efficacy in preventing human clinical disease in household (consumer) environments. However, as was noted above, there are a number of publications that describe the efficacy in other use-environments. Finally, the literature search did not reveal any evidence that--despite the long history of chloroxylenol use world-wide in household environments—either antimicrobial or antibiotic resistance was linked to exposures in homes, industries, or the environment.

Chloroxylenol Mechanism-of-Action

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Appendix B

Assessment of the Developmental and Reproductive (DART) Database for Chloroxylenol

Appendix B: Assessment of the Developmental and Reproductive (DART) Database for Chloroxylenol

Summary of Findings

Three rat developmental toxicity studies of chloroxylenol are available. These studies establish developmental no-observed-effect levels (NOELs) of 300 and 1,000 mg/kg/day. No rabbit developmental toxicity studies were identified. However, because the one rat teratology study (Siglin 1991) has been characterized by FDA as "adequately characterizes chloroxylenol's potential effects on embryo and fetal development," FDA should not require a rabbit study in this circumstance.

In addition to the three rat developmental toxicity studies, a multi-generation rat reproduction study (Harr 1978) is also available and has been summarized in a review by Guess and Bruch (1986). Although the adequacy of this study cannot be determined based on the limited information provided in the review, this study should be considered by FDA in order to fully characterize chloroxylenol's potential to cause development and reproduction toxicity.

FDA DART Requirements

The U.S. Food and Drug Administration (FDA) follows the International Conference on Harmonisation (ICH) guidelines for the detection of toxicity to reproduction and development (ICH 1994). This guideline recommends that observations be conducted "through one complete life cycle, i.e., from conception in one generation through conception in the following generation," to detect both immediate and delayed effects. While flexibility in testing approach is emphasized, a three-study design is generally employed to address all life stages. These include:

1. **A study of fertility and early embryonic development.** This type of study is generally conducted in rats. It involves exposure of females from at least 2 weeks prior to mating until some point mid-gestation (after implantation), and exposure of males for at least 4 weeks prior to mating until sometime after mating (usually until the pregnancy status of the mated females has been determined). The primary goals are to assess effects on the maturation of gametes in both sexes, mating behavior, fertility, implantation, and the pre-implantation stages of the embryo.
2. **A study for effects on prenatal and postnatal development.** This type of study is generally conducted in rats. It typically involves the exposure of females from the point of implantation (usually gestational day [GD] 6) through the period of lactation. Offspring are delivered, and at the point of weaning (the end of lactation), at least one male and one female of each litter

is selected for rearing until adulthood to assess subsequent reproductive performance. The primary goals are to assess effects on pregnancy, and pre- and post-natal growth and development of the offspring, and any functional defects in the offspring related to behavior, maturation, and reproduction.

3. **A study for effects on embryo-fetal development.** This type of study is generally conducted in two species: the rat and the rabbit. Exposure typically occurs during the period of major organogenesis (from the point of implantation until closure of the hard palate), but may be extended until the end of gestation, just prior to birth. The primary goal of this type of study is to assess adverse effects on pregnancy and the developing offspring. In particular, the resulting fetuses are examined for external, visceral, and skeletal anomalies in a study of embryo-fetal development.

Specific details regarding recommendations and requirements for the conduct of these studies can be found in the ICH-S5A guideline (ICH, 1994).

Chloroxylenol DART Database

Only one study has been identified by FDA as providing data relevant to assessing DART (U.S. FDA 2013). In their review, FDA stated that this study is adequate for characterizing chloroxylenol's potential effects on embryo and fetal development, but they concluded that "additional studies are necessary to assess the effect of chloroxylenol on fertility and early embryonic development and on pre- and postnatal development." Our review of the literature identified four additional studies, the availability of which was not acknowledged by FDA, but that should be considered by FDA in its evaluation of DART for chloroxylenol. The chloroxylenol DART studies identified in this effort are shown in Table B1. Each of these is discussed further below.

FDA's recent review of the available safety data for chloroxylenol (U.S. FDA 2013) addressed only one study relevant to assessing DART. This single study reviewed by FDA is a rat teratology study conducted at Springborn Laboratories on behalf of Nipa Laboratories, Inc. (Siglin 1991; FDA citation 186). It generally follows the guidelines of a study for effects on embryo-fetal development, as described above. In this study, groups of 25 mated female Sprague-Dawley rats were treated by oral gavage with 0, 100, 500, or 1000 mg/kg/day of chloroxylenol on GDs 6–15. The test substance was administered in a corn-oil vehicle at a constant volume of 10 mL/day. Based on reduced food consumption and body-weight gains observed at 500 and 1,000 mg/kg/day, the study investigators determined that the maternal no-observed-effect level (NOEL) was 100 mg/kg/day. Based on the lack of evidence of any embryo-fetal effects, the study investigators determined that the NOEL for developmental toxicity was 1000 mg/kg/day, the highest dose tested. FDA concurs with these NOAELs and states that this study "adequately characterizes chloroxylenol's potential effects on embryo and fetal development" (U.S. FDA 2013). This study has also been reviewed by the U.S. Environmental Protection Agency (EPA) in its reregistration eligibility decision (RED) document for chloroxylenol (U.S. EPA 1994) and in the Agency's more recent summary of

human health effects data for chloroxylenol (U.S. EPA 2009a), which generally concurred with FDA on the maternal and developmental NOELs.

Table B1. Available chloroxylenol DART studies

Study Type	Reference	Other Identifying Information
Rat teratology study	Siglin JC. 1991. 4 Chloro 3,5 Xylenol (PCMX) Teratology Study in Rats. Springborn Laboratories, Inc. Nipa-1991-4. July 10, 1991.	US FDA reference 186 US EPA MRID 42002702
Rat teratology study	Noda T, S Morita, A Yamada, S Ohgaki. 1983. Safety evaluation for use in household products (IV). Teratological studies on p-chloro-m-xylenol in rats. Annual Report of the Osaka City Institute for Public Health and Environmental Sciences 45:100-105.	---
Rat teratology study	[Citation unknown]. (Mentioned in U.S. EPA 2009a,b)	US EPA MRID 42002701
Rat reproductive study	Harr JR. 1978. Pennwalt Corporation. (Discussed in Guess and Bruch 1986)	---
Chicken teratology study	Jain PK, PC Gangwar. 1972. Effects of storage and antibiotic treatments on developmental malformations in chickens. Indian Journal of Experimental Biology 10:319-321.	---

Another teratology study of chloroxylenol in rats has been described in the Japanese literature (Noda et al. 1983), a translation of which was provided to Exponent for review by the American Cleaning Institute. In this study, groups of 24–29 mated female Wistar rats were treated by oral gavage with 0, 100, 300, or 900 mg/kg/day of chloroxylenol on GDs 0–19 (16–19 dams per group sacrificed on GD 20 for examination of the offspring prior to birth) or treated from GD 0 until birth (8–10 dams per group that were allowed to give birth, with examination of offspring on postnatal day 21). The test substance was administered in an olive-oil vehicle at a constant volume of 10 mL/day. Food consumption was significantly reduced on certain gestational days in both the mid- and high dose groups, and body-weight gain was reduced in the high-dose group. Clinical signs of toxicity, some deaths (n=4), and total litter resorptions were also observed at the high dose; however, the mean number of fetuses per litter did not differ across treatment groups. Based on these findings, Exponent considers the maternal NOEL to be 100 mg/kg/day, identical to that observed in the Siglin (1991) study described above. In dams from the high-dose group sacrificed on GD 20, fetal body weights were significantly decreased, and ossification of the sternebrae and vertebrae was found to be delayed. There were no treatment-related malformations. Based on these data, Exponent considered the developmental NOEL to be 300 mg/kg/day. However, the offspring of dams that were allowed to deliver showed no effects of gestational treatment on postnatal growth or the attainment of certain maturational markers (ear detachment, hair growth, and eyelids opening), indicating that the offspring findings prior to birth are likely due to developmental delay, subsequent to maternal toxicity.

Although the Noda et al. (1983) study suggests a lower developmental NOEL (300 mg/kg/day) than the Siglin (1991) study (1000 mg/kg/day), certain differences between these two studies must be kept in mind. First, these studies were conducted in two different strains of rats, which

might exhibit differing susceptibilities. Further, dosing was done throughout gestation (GD 0–19 or GD 0 to birth) in the study by Noda et al. (1983), while dosing in the Siglin (1991) study was only from GD 6 through GD 15. Finally, although the developmental NOEL in the Noda et al. (1983) study was lower than that in the Siglin (1991) study, observation of the offspring during the postnatal period showed that the findings upon which the developmental NOEL was based were reversible after birth.

In addition to these two studies, another rat teratology study was mentioned in EPA's recent summary of human health effects data for chloroxylenol (U.S. EPA 2009a), although a full citation for this study was not provided. This study is a supplementary range-finding rat developmental study in which Sprague Dawley rats were administered 0, 150, 300, 750, or 1000 mg/kg/day of chloroxylenol on GD 6-15. EPA considered the NOEL for maternal toxicity to be 750 mg/kg/day based on reduced body weight gains. EPA considered the developmental NOEL to be 1000 mg/kg/day.

A review article summarizing the toxicity data available in the mid-1980s for chloroxylenol (Guess and Bruch 1986) mentions a rat reproductive study that was planned for examination of four successive generations of animals, but was discontinued after only two generations. This study is identified as one of the "Penwalt Studies" (Harr 1978), and at the time the article was published, it was part of FDA Docket No. 75N-0183. However, this study was not reviewed in FDA's recent review of the available safety data for chloroxylenol (U.S. FDA 2013). As summarized by Guess and Bruch (1986), in this study, groups of rats were exposed by oral gavage to 0.75% chloroxylenol (75 mg/kg/day), 0.24% chloroxylenol (24 mg/kg/day), 0.08% chloroxylenol (8 mg/kg/day), 3 mL of fresh soap (3.75% chloroxylenol; 112.5 mg/kg/day), 3 mL of fresh soap without chloroxylenol, 0.3 mg of hexachlorophene, or vehicle (50% propylene glycol) beginning 100–150 days prior to mating. The specific strain of rat and number of animals per group were not identified. The resulting F_1 pups were necropsied for examination (the day of sacrifice was not identified) or selected for mating at 120–150 days of age (following direct dosing beginning at weaning). Although not reported, standard protocol would be for the maternal animals to be dosed with the test substances throughout the premating, mating, gestation, and lactation periods. Parameters reported to have been assessed in this study include "litter size, percent conception, total number of pups per female bred, percent mortality, litter weight, average weight per pup, sex ratio, and gross pathologic appearance." Reduced F_1 and F_2 litter sizes and numbers of pups per female bred were reported at 75 mg/kg/day of chloroxylenol. How these two parameters differ from one another, however, is not clear, and it is likely that they reflect the same finding. Those receiving fresh soap were also reported to have a reduced number of pups per female bred.

Without the original study report to review, the adequacy of this study for assessing effects on fertility, reproduction, and postnatal development cannot be determined, and specific NOELs cannot be called out. However, according to Guess and Bruch (1986), no adverse findings were reported at a dose below 75 mg/kg/day.

The only other available study to address the potential developmental toxicity of chloroxylenol is a study involving exposure of chicken eggs to a solution containing 0.048% chloroxylenol (i.e., a 1% Dettol solution, which contains 4.8% chloroxylenol), with subsequent examination of the chicken embryos for malformations (Jain and Gangwar 1972). Dettol exposure resulted in

an incidence of malformations similar to that observed in the untreated control group. Despite these findings, this study is considered inadequate to assess the potential developmental toxicity of chloroxylenol to mammalian species.

DART Assessment

The following conclusions were reached in reviewing the available chloroxylenol DART database. The available studies established developmental NOELs of 300 and 1,000 mg/kg/day. FDA indicated that the one rat study (Siglin 1991) is adequate for characterizing chloroxylenol's potential effects on embryo and fetal development. Further, EPA concluded that the available developmental studies were sufficient to "support chloroxylenol uses and the basic Tier 1 toxicity data requirements" (U.S. EPA 2009b). With regard to the three-study design generally employed to address all life stages:

- **A study of fertility and early embryonic development.** The Pennwalt study (Harr 1978) assessed effects on reproduction through two generations of animals. However, it was not reported that this study specifically evaluated effects on the maturation of gametes; it is possible that such data were collected but not addressed in the review by Guess and Bruch (1986). Additionally, details provided in the review by Guess and Bruch (1986) regarding the conduct of this study are not sufficient to assess its adequacy. Nevertheless, it is possible that the Pennwalt study may provide data that are sufficient to characterize chloroxylenol's potential effects on fertility and early embryonic development.

- **A study for effects on prenatal and postnatal development.** The two (possibly three) available rat teratology studies likely cover the requirement for an assessment of prenatal development. The Pennwalt study (Harr 1978) assessed effects of chloroxylenol on early postnatal development. It does not appear, however, that this study examined certain functional endpoints in the offspring (i.e., the maturation of developmental landmarks or behavior). Further, the adequacy of this study cannot be determined based on the limited information provided in the review by Guess and Bruch (1986). Although the study by Noda et al. (1983) examined offspring from birth until weaning on postnatal day 21, the maternal animals were not dosed during the postnatal period; further, minimal functional endpoints were assessed in the offspring. Therefore, the study of Noda et al. (1983) does not fulfill the general requirements for an assessment of postnatal development. Overall, the Pennwalt study, in combination with the available rat teratology studies, provide data that should be considered in order to characterize chloroxylenol's potential effects on prenatal and postnatal development.

- **A study for effects on embryo-fetal development.** Three studies in rats that assess the effects of chloroxylenol on embryo-fetal development were identified. However, no studies in rabbits were found. The one rat teratology study (Siglin 1991) has been characterized by FDA as "adequately

characterizes chloroxylenol's potential effects on embryo and fetal development." As such, FDA should not require a rabbit study in this circumstance.

Additional Points for Consideration

FDA determined that the available studies were inadequate based on a limited understanding of the available literature. The adequacy of the data available in the Pennwalt study (Harr 1978) cannot be determined from the information available to this reviewer. If this study is sufficient to assess the effects of chloroxylenol on fertility and postnatal development, then additional studies are not required. As noted previously, the ICH guideline for the detection of toxicity to reproduction and development emphasizes flexibility in its testing approach. Therefore, although two separate studies are typically done to fulfill requirements 1 and 2 above, it is possible to design a single study to obtain the necessary data if additional studies are deemed to be required. Such a study would take the form of an extended one-generation reproduction study, in which dosing of females would take place from at least two weeks prior to mating and continue through to weaning on lactational day 21. Males would also be exposed from at least four weeks prior to mating until sometime after the pregnancy status of the females had been determined; these animals would be assessed for effects on mating, fertility, and spermatogenesis. The females would be evaluated for effects on mating, fertility, and sexual maturation. The resulting offspring would be assessed for functional deficits (including assessments of behavior and sexual maturation, at a minimum), and at least one male and one female per litter would be selected for rearing until adulthood for subsequent reproductive assessment.

The ICH guideline recommends that the dosing in these types of studies should typically be done using the same route(s) of exposure intended for humans. Because chloroxylenol is a topical antimicrobial product, this suggests that test substance administration should be by the dermal route. The studies reviewed herein, however, used the oral route for test substance administration. The benefit of dosing via the oral route is that better control of doses can be achieved. Dermal application in some studies can be misleading, because animals incur an additional oral dose while grooming, hence resulting in inaccurate estimates of dose from dermal exposure. For any future studies, it will be important to confirm the best route of exposure during the study design process.

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Appendix C

Assessment of the Hormonal Effects Database for Chloroxylenol

Appendix C: Assessment of the Hormonal Effects Database for Chloroxylenol

Summary of Findings

Ten *in silico/in vitro* studies that assess chloroxylenol's ability to interact with the estrogen receptor (nine studies) and the androgen receptor (one study) are available in the open literature (Table C1). In all cases, chloroxylenol was found to have moderately weak receptor activity. Consequently, it is likely that relatively high concentrations of the compound would be required to induce a relevant effect in an intact animal.

In addition to the above ten studies, numerous *in vivo* studies conducted to fulfill regulatory requirements are available that assess chloroxylenol's activity in the intact animal (Table 1). Overall, these *in vivo* studies show that chloroxylenol has no effect on hormonally sensitive endpoints, including the weights and histopathology of hormonally sensitive tissues and the expression of hormonally sensitive parameters in DART studies. Doses administered in at least some of these studies reached the generally accepted limit dose of 1,000 mg/kg/day.

Taken together, the available data indicate that although chloroxylenol may demonstrate weak receptor activity in *in vitro* test systems, results from *in vivo* studies demonstrate that the hormonal activity of this chemical is too weak to interact at the estrogen or androgen receptor to induce hormonal effects in the intact animal.

The studies listed in Table 1 support these conclusions and should be considered by the FDA in a full weight-of-evidence assessment of the hormonal potential of chloroxylenol.

Table C1. Available studies for evaluation of the hormonal effects of chloroxylenol

Study	Other Identifying Information
***In silico* and *in vitro* studies published in the open literature**	
Georgieva S, Y Koleva. 2011. Metabolic estrogenic activity of some endocrine disruptor chemicals. Pharmacia LVIII:65-76.	---
Houtman CJ, AM Van Oostveen, A Brouwer, MH Lamoree, J Legler. 2004. Identification of estrogenic compounds in fish bile using bioassay-directed fractionation. Environmental Science and Technology 38:6415-6423.	---
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Miller D, BB Wheals, N Beresford, JP Sumpter. 2001. Estrogenic activity of phenolic additives determined by an in vitro yeast bioassay. Environmental Health Perspectives 109:133-138.	---

Study	Other Identifying Information
Mombelli E. 2011. Evaluation of the OECD (Q)SAR application toolbox for the profiling of estrogen receptor binding affinities. SAR and QSAR in Environmental Research 23:37-57.	---
Nakama A, K Funasaka, M Shimizu. 2007. Evaluation of estrogenic activity of organic biocides using ER-binding and YES assay. Food and Chemical Toxicology 45:1558-1564.	---
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***In vivo* studies conducted for regulatory purposes**	
Cappetti N. 1976. 30-day Subacute Toxicity of PCMX in Rats: Summary Interim Report. Experiment 80N. Pennwalt Corporation.	U.S. EPA MRID 40223109. (Of unknown relevance to assessing hormonal effects.)
Chesterman H, PN Whitehead, AE Street. 1973a. RBA 666. Oral Toxicity Studies in Beagle Dogs. Initial Studies. Huntington Research Centre. RKT43/73674. September 7, 1973.	---
Chesterman H, R Heywood, MH Barber, AE Street, CP Cherry. 1973b. RBA 666. Oral Toxicity Studies in Beagle Dogs. Repeat Dosage for 13 Weeks. Huntington Research Centre. RKT44/73693. December 31, 1973.	---
Davies RE, M Liggett, A Street, R Heywood, C Cherry, P Gallagher. 1974. Effects of Repeated Applications of Dettol RBA 666 to the Vaginal Mucosa of Rabbits for Two Weeks. Huntington Research Centre.	Discussed in CIR, 1985.
Doyle RL, JR Elsea. 1965. Subacute and Chronic Dermal Application of Ottasept Extra to Rabbits. Hill Top Research, Inc. P-13. November 3, 1965.	U.S. EPA MRID 00066995.
Harr JR. 1978. Pennwalt Corporation.	Discussed in Guess and Bruch, 1986; noted as previously being in U.S. FDA Docket No. 75N-0183.
Hunter B, JL Bridges, AJ Newman. 1973a. Dettol RBA 666. Preliminary Assessment of Toxicity to Rats. Oral Administration for Four Weeks. Huntington Research Centre.	Discussed in CIR, 1985.
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Study	Other Identifying Information
Morris TD. 2002. A 13-Week Dermal Toxicity Study of PCMX in Mice. WIL Research Laboratories, Inc. WIL-304003. Clariant Corporation. July 2, 2002.	Identified in U.S. FDA, 2013b as US FDA reference 185, data of relevance to carcinogenic potential.
Momma J, K Takada, Y Aida, H Yoshimoto, K Naito, Y Suzuki, Y Nakaji, Y Kurokawa, M Tobe. 1988. Combined long-term toxicity and carcinogenicity test of p-chloro-m-xylenol (PCMX) applied to female mouse skin. Eisei Shikenjo Hokuku 63:39-47.	Japanese.
Noda T, S Morita, A Yamada, S Ohgaki. 1983. Safety evaluation for use in household products (IV). Teratological studies on p-chloro-m-xylenol in rats. Annual Report of the Osaka City Institute for Public Health and Environmental Sciences 45:100-105.	Japanese.
Siglin JC. 1991. 4 Chloro 3,5 Xylenol (PCMX) Teratology Study in Rats. Springborn Laboratories, Inc. Nipa-1991-4. July 10, 1991.	Identified in U.S. FDA, 2013b as U.S. FDA reference 186, data of relevance to DART; US EPA MRID 42002702.
[90-day dermal toxicity study in rabbits]	U.S. EPA MRID 40223124. (Of unknown relevance to assessing hormonal effects.)
[90-day dermal toxicity study in and mice]	U.S. EPA MRID 46092002. (Of unknown relevance to assessing hormonal effects.)
[90-day dermal toxicity study in and mice]	U.S. EPA MRID 46030401. (Of unknown relevance to assessing hormonal effects.)
[Rat developmental toxicity study]	U.S. EPA MRID 42002701.

FDA Requirements

At present, the U.S. Food and Drug Administration (FDA) has issued only draft guidance for assessing the endocrine potential of drugs (U.S. FDA 2013a). This guidance indicates that the typical nonclinical studies that can be used to address hormonal effects include:

- Receptor-binding and enzyme assays
- Pharmacology studies
- Repeat-dose toxicity studies
- Developmental and reproductive toxicity studies
- Carcinogenicity studies.

With regard to receptor-binding assays, the guidance notes that these assays "serve only as an initial screening device," and that the lack of binding does not rule out a possible endocrine effect. Further, receptor binding does not necessarily translate to a hormonal effect in an intact organism, but does suggest that additional testing may be necessary to fully characterize the observed response.

With regard to the last three study types listed (repeat-dose toxicity studies, developmental and reproductive toxicity studies, and carcinogenicity studies), these are typical nonclinical studies conducted to assess the safety of new drugs. The guidance points out that these studies include a variety of endpoints that may be helpful for identifying potential endocrine effects. For repeat-dose studies, these include "changes in body weight, organ weights, gross organ pathology, clinical chemistry, and histopathology." Developmental and reproductive toxicity studies are identified as being "particularly suited for detecting endocrine effects," and include assessment of fertility and reproduction, as well as endpoints related to both prenatal and post-natal development. Finally, carcinogenicity studies assess many of the same endpoints as addressed in repeat-dose toxicity studies, but also include evaluation of tumors that may be endocrine-related.

Chloroxylenol Hormonal Effects Database

In FDA's recent review of the safety data available for chloroxylenol (U.S. FDA 2013b), no studies that addressed the potential hormonal effects of chloroxylenol were identified. However, a detailed search has identified several studies that provide relevant information on the potential for hormonal effects from chloroxylenol. These include studies of *in silico* quantitative structure-activity relationships (QSAR) modeling and *in vitro* receptor binding or transactivation studies published in the open literature. In addition, mammalian toxicology studies conducted to fulfill regulatory requirements provide additional information regarding possible hormonal effects in intact animals.

Ten published *in silico* and *in vitro* studies conducted between 2000 and the present were identified in this effort. These are summarized below and listed in Table C2. Together, these studies provide important information regarding the potential hormonal activity of chloroxylenol, and should be considered by FDA in their evaluation.

Three *in silico* and six *in vitro* assays evaluated the estrogenic activity of chloroxylenol. Using two different QSAR programs, chloroxylenol was predicted to show weak estrogen receptor binding activity (Klopman and Chakravarti 2003; Georgieva and Koleva 2011). Another QSAR study predicted that chloroxylenol would not bind to the estrogen receptor and was considered a false positive (Mombelli 2011). The reason that the results of the latter study differ from those of the other two QSAR studies is not clear, but may have to do with limitations of the ER profiler program to detect weak receptor binders. The prediction of weak estrogen activity was verified in the *in vitro* studies, which showed that chloroxylenol binds weakly to the human estrogen receptor and can activate the receptor in both yeast transactivation assays and mammalian cell systems (Nishihara et al. 2000; Nakano et al. 2002; Miller et al. 2001; Nakama et al. 2007; Houtman et al. 2004; Terasaka et al. 2006). An additional *in vitro* study demonstrated that chloroxylenol possesses weak anti-androgenic activity as well (Rostkowski et al. 2011). The results of this single latter study, however, do not provide an adequate basis for forming conclusions regarding the anti-androgenic activity of chloroxylenol.

Table C2. Available published chloroxylenol studies on hormonal effects, in chronological order

Study Type	Reference	General findings
Estrogen Receptor (ER) Studies		
Yeast two-hybrid ER assay[1]	Nishihara et al., 2000.	Chloroxylenol was identified as a weak ER transactivator with a REC_{10} of 10^{-4} M, with the REC_{10} defined as 10% of the agonist activity of 10^{-7} M 17β-estradiol.
Yeast ER transactivation assay	Miller et al., 2001.	Chloroxylenol was found to have estrogenic activity equal to 1/900,000 that of 17β-estradiol.
Yeast two-hybrid ER assay[1]	Nakano et al., 2002.	Chloroxylenol was identified as a weak ER transactivator with an REC_{10} of 2×10^{-5} M, with the REC_{10} defined as 10% of the agonist activity of 10^{-7} M 17β-estradiol. Cytotoxicity to the yeast was noted at 10^{-4} – 10^{-3} M.
QSAR study	Klopman and Chakravarti, 2003.	Chloroxylenol was predicted to have an RBA of 0.0445 using the MultiCASE program, with the RBA defined as "100 times the ratio of the molar concentrations of [^{3}H]estradiol and the competing chemical required to decreased the receptor bound radioactivity by 50%."
Human ER transactivation assay	Houtman et al., 2004.	Chloroxylenol was identified in the deconjugated nonpolar residual extract of bream fish bile that exhibited estrogenic activity. Chloroxylenol was found to be weakly estrogenic in the ER-CALUX transactivation assay with an EEF of 2.35×10^{-7} M, with the EEF defined as the ratio of the EC_{50} of 17β-estradiol divided by the EC_{50} of the test substance; cytotoxicity noted at $\geq 5 \times 10^{-5}$ M.
Estrogen-responsive cDNA microarray assay (EstrAssay)	Terasaka et al., 2006.	Chloroxylenol treatment of human MCF-7 breast cancer cells was found to show "low but distinct correlations" with the expression of estrogen responsive genes (R value = 0.26); correlations were primarily with genes related to cell proliferation.
Yeast ER transactivation assay (YES) and human ER binding assay	Nakama et al., 2007.	In the YES assay, chloroxylenol in the presence and absence of metabolic activation (rat S9 fraction) was judged to be pseudo-positive, because it induced receptor transactivation at only one concentration with no dose-response; 50% growth inhibition was evident at 53 µg/mL. In the human ER binding assay, chloroxylenol was found to have an IC_{30} of 14 µg/L and a RBA of 5×10^{-5} % in the human ER binding assay, with the RBA defined as 100× the ratio of the IC_{30} of 17β-estradiol divided by the IC_{30} of the test substance.
QSAR study	Georgieva and Koleva, 2011.	Chloroxylenol was predicted to be a weak ER binder using the OECD QSAR application toolbox; one hydroxylated metabolite was predicted to be a moderate ER binder.
QSAR study	Mombelli, 2011	Chloroxylenol was predicted to not binding the ER using the ER profiler application in the OECD QSAR toolbox.
Androgen Receptor (AR) Studies		
Yeast AR transactivation antagonism assay (anti-YAS) and human AR transactivation antagonist assay (AR-CALUX)	Rostkowski et al., 2011.	The deconjugated HPLC fractions of trout fish bile containing high concentrations of chloroxylenol exhibited anti-adnrogenic activity. Chloroxylenol was found to have potency of 0.16 relative to the reference standard flutamide in the anti-YAS assay; apparently not evaluated in AR-CALUX assay.

[1]Includes expression plasmids for the ERα ligand-binding domain and TIF_2 coactivator.

AR = androgen receptor; EC_{50} = 50% effective concentration; EEF = estradiol equivalency factor; ER = estrogen receptor; HPLC = high performance liquid chromatography; IC_{30} = 30% inhibitory concentration; RBA = relative binding affinity; REC_{10} = 10% relative effective concentration; QSAR = quantitative structure activity relationship.

While these ten studies show that chloroxylenol possesses the capability to interact at the estrogen (and possibly the androgen) receptor(s), it is not established that results from the *in silico* and *in vitro* studies are predictive of the activity of this compound in an *in vivo* system. In all of the available *in silico* and *in vitro* studies, chloroxylenol was found to have moderately weak receptor activity. Consequently, it is likely that relatively high concentrations of the compound would be required to induce a measurable effect in an intact animal. Whether such concentrations could be achieved internally remains unknown.As noted in FDA's draft guidance for assessing the endocrine potential of drugs (U.S. FDA 2013a), examination of the results from studies conducted to fulfill regulatory requirements provides an indication of whether chloroxylenol exposure alters the expression of parameters that may be hormonally sensitive in the intact animal. For example, in subchronic and chronic exposure studies, the weights and histopathology of various hormonally sensitive tissues are frequently evaluated endpoints. Carcinogenicity studies also assess endocrine-related tumor development. Additionally, developmental and reproductive toxicity studies generally assess hormonally sensitive processes such as fertility, reproduction, and the development of genitalia. The subchronic and chronic studies of chloroxylenol that were identified as providing information on hormonally sensitive parameters are detailed in Table 3. The reproductive and developmental studies of chloroxylenol that were identified as providing information on hormonally sensitive parameters are detailed in Table 4. It should be noted that the tabulated information for many of these studies was derived from two review articles on chloroxylenol (CIR 1985; Guess and Bruch 1986). It is possible that additional hormonally sensitive parameters besides those detailed in Tables 3 and 4 were assessed in these studies but not discussed in the reviews.

Other toxicity studies of chloroxylenol detailed in these reviews and in the U.S. Environmental Protection Agency (EPA) reregistration eligibility decision document for chloroxylenol (U.S. EPA 1994), but for which the assessment of hormonally sensitive parameters could not be confirmed, are listed in Table 5. It is possible that these studies also contain information on hormonally sensitive parameters that would be relevant to an assessment of the hormonal potential of chloroxylenol. Finally, it should also be noted that the 2009 EPA registration review preliminary work plan for chloroxylenol (U.S. EPA 2009a), and as well, EPA's more recent summary of human health effects data for chloroxylenol (U.S. EPA 2009b) list 90-day dermal toxicity studies in rabbits (U.S. EPA MRID 40223124) and mice (U.S. EPA MRID 46092002 and U.S. EPA MRID 46030401), a 90-day rat oral toxicity study (U.S. EPA MRID 40223124), and an additional rat developmental toxicity study (U.S. EPA MRID 42002701); a 6-month oral toxicity study in rats is also mentioned, but an EPA MRID number for this study is not provided. Full citations for these studies are not available, and it is possible that these studies may be the same as some of those already detailed in Tables C3–C5. These studies additionally should be considered by FDA in their assessment of chloroxylenol's potential to cause hormonal effects.

Table C3. Available chloroxylenol subchronic and chronic studies with evaluation of hormonally sensitive parameters

Study Type	Reference	Doses and Hormone-sensitive Endpoints Assessed
Subacute/Subchronic Studies		
13-week dermal toxicity study in mice	Morris, 2002. (US FDA reference 185)	*Doses:* 0%, 15%, 30% and 60% chloroxylenol solutions in acetone applied to skin (clipped of hair) daily for 13 weeks. Approximate doses were 0, 250, 500, and 1000 mg/kg/day. *Relevant endpoints:* Weights of the adrenals, epididymides, ovaries with oviducts, testes, thyroid with parathyroids, and uterus; histopathology of the adrenal glands, epididymides, mammary glands (females), ovaries and oviducts, pituitary, prostate, seminal vesicles, testes, thyroids (with parathyroids when present), uterus with cervix, and vagina. *Findings:* No treatment-related effects on weights or histopathology of relevant organs.
4-week oral toxicity study in rats	Hunter et al., 1973a.	*Doses:* 5 mL/kg/day of a 0%, 25%, 50% or 100% solution of Dettol.[1] Approximate doses were 0, 60, 120, and 240 mg/kg/day.[2] *Relevant endpoints:* Weights of the adrenals, ovaries and testes.[2] *Findings:* No treatment-related effects on weights of relevant organs.[2]
13-week oral toxicity study in rats	Hunter et al., 1973b.	*Doses:* 0, 0.5 mL/kg/day of a 5% emulsion of Dettol, 5.0 mL/kg/day of a 25% emulsion of Dettol, or 5.0 mL/kg/day of a 50% emulsion of Dettol daily for 13 weeks. *Relevant endpoints:* Weights of the adrenals, ovaries, pituitary, testes and uterus; histopathology of the adrenals, mammary glands, ovaries, pituitary, prostate, seminal vesicles, testes, thyroid and uterus. *Findings:* No treatment-related effects on weights or histopathology of relevant organs.
14-day vaginal application study in rabbits	Davies et al., 1974.	*Doses:* 1 mL/kg/day of 0%, 10%, or 25% solution of Dettol.[2] *Relevant endpoints:* Histopathology of the vaginal mucosa.[2] *Findings:* No treatment-related effects on vaginal histology.[2]
4-week oral toxicity study in dogs	Chesterman et al., 1973a.	*Doses:* 2.0 mL/kg/day of an undiluted solution of Dettol for 4 weeks, 4.0 mL/kg/day of a undiluted solution of Dettol for 4 weeks following by 5 mL/kg/day of a 50% solution of Dettol for another 4 weeks, or 8.0 mL/kg/day of an undiluted solution of Dettol for up to 3.5 weeks. *Relevant endpoints:* Weights of the adrenals, gonads, pituitary, prostate, thyroids, and uterus. *Findings:* No treatment-related effects on weights of relevant organs.
13-week oral toxicity study in dogs	Chesterman et al., 1973b.	*Doses:* 0, 0.5 mL/kg/day of a 25% solution of Dettol, 5.0 mL/kg/day of a 25% solution of Dettol, or 5.0 mL/kg/day of a 50% solution of Dettol. *Relevant endpoints:* Weights of the adrenals, gonads, pituitary, prostate, thyroids, and uterus; histopathology of the adrenals, gonads, mammary glands, pituitary, prostate, thyroids, and uterus. *Findings:* No treatment-related effects on weights or histopathology of relevant organs.

Study Type	Reference	Doses and Hormone-sensitive Endpoints Assessed
Chronic Studies		
79-week dermal carcinogenicity study in female mice	Momma et al. 1988.	*Doses:* 0%, 1%, and 10% chloroxylenol solutions in ethanol applied to shaved skin twice weekly for 79 weeks. *Relevant endpoints:* Histopathology of the ovaries, pituitary, adrenals, thyroid gland, and uterus. *Findings:* No treatment-related neoplastic or non-neoplastic changes in these tissues were evident.
Subacute (3-week) and Subchronic (13-week) dermal study in rabbits	Doyle and Elsea 1965. (US EPA MRID 00066995)	*Doses:* 1 mL/kg/day of 0%, 1.8% or 18% Ottasept Extra (chloroxylenol) in propylene glycol applied to clipped intact or abraded skin 5 times per week for 3 or 13 weeks. *Relevant endpoints:* Histopathology of the adrenals, ovaries, testes and uterus. *Findings:* No treatment-related effects on the histopathology of relevant organs.

[1]Dettol contains 4.8% chloroxylenol, 10% alcohol, and 20% terpineol in a castor-oil soap base.

[2]Information as provided in Guess and Bruch, 1986.

Table C4. Available chloroxylenol DART studies with evaluation of hormonally sensitive parameters

Study Type	Reference	Doses and Hormone-sensitive Endpoints Assessed
Developmental Studies		
Rat teratology study	Siglin, 1991. (US FDA reference 186; US EPA MRID 42002702)	*Doses:* 0, 100, 500, and 1000 mg/kg/day by oral gavage on GD 6-15. *Relevant endpoints:* Number of implantations, incidences of live/dead fetuses, resorptions, external anomalies of the genitalia. *Findings:* No treatment-related effects on any of the above parameters.
Rat teratology study	Noda et al., 1983.	*Doses:* 0, 100, 300, and 900 mg/kg/day by oral gavage on GD 0-19 or GD 0 to birth. *Relevant endpoints:* Number of implantations, incidences of live/dead fetuses/pups, external anomalies of the genitalia. *Findings:* No treatment-related effects on any of the above parameters with the exception of increased late resorptions (dead fetuses) in high dose dams sacrificed on GD 20, but not effect on numbers of live fetuses (resorptions thought to be an indication of maternal toxicity).
Reproductive Studies		
Rat reproductive study	Harr, 1978.	*Doses:* 0%, 0.75% chloroxylenol (75 mg/kg/day), 0.24% chloroxylenol (24 mg/kg/day), 0.08% chloroxylenol (8 mg/kg/day), 3 mL of fresh soap (3.75% chloroxylenol; 112.5 mg/kg/day), 3 mL of fresh soap without chloroxylenol, 0.3 mg of hexachlorophene, or vehicle (50% propylene glycol) beginning 100-150 days prior to mating through two generations of animals.[1] *Relevant endpoints:* Litter size, percent conception, total number of pups per female bred, gross pathology of the genitalia.[1] *Findings:* No treatment-related effects on any of the above parameters.[1]

GD = gestation day

[1]Information as provided in Guess and Bruch, 1986; this study was planned for the production of four generations of rats, but was terminated after the production of only two generations due to a change in corporate direction.

Table C5. Other available chloroxylenol studies for which evaluation of hormonally sensitive parameters cannot be confirmed

Study Type	Reference
Subacute/Subchronic studies	
30-day toxicity study in rats	Cappetti, 1976 (US EPA MRID 40223109; as cited in US EPA, 1994)
30-day oral toxicity study in rats	Harr, 1978 (as cited in Guess and Bruch, 1986)
90-day oral toxicity study in rats	Harr, 1978 (as cited in Guess and Bruch, 1986)
30-day dermal toxicity study in rabbits	Harr, 1978 (as cited in Guess and Bruch, 1986)
Chronic studies	
1-year dermal toxicity study in dogs[1]	Harr, 1978 (as cited in Guess and Bruch, 1986)

[1]Reported to have been terminated after 8 months due to a change in corporate direction.

The above tabulated *in vivo* mammalian toxicity studies (Tables 3–5) provide considerable data relevant to the question of potential hormonal effects from exposure to chloroxylenol. The subacute/subchronic and chronic studies show no effects of chloroxylenol exposure on the weights and histopathology of hormonally sensitive tissues. Additionally, the DART studies do not indicate an effect of chloroxylenol exposure during gestation or prior to mating on any of the hormonally sensitive parameters measured in these studies. It should be noted further that, in at least some of these studies, treatment involved oral dosing at 1,000 mg/kg/day, the generally accepted limit dose for safety toxicity studies.

Hormonal Effects Assessment

Review of the available database regarding the potential hormonal effects of chloroxylenol supports the following conclusions:

1. ***In silico* and *in vitro* data.** The ten studies detailed in Table 2 generally show that chloroxylenol possesses a capability to interact at the estrogen (and possibly the androgen) receptor(s), outside of an intact animal. In all cases, however, chloroxylenol was found to have moderately weak receptor activity. Consequently, it is likely that relatively high concentrations of the compound would be required to induce a relevant effect in an intact animal.

2. ***In vivo* mammalian data.** The studies tabulated in Tables 3 and 4 consistently show that chloroxylenol exposure in the intact animal has no effect on hormonally sensitive endpoints, including the weights and histopathology of hormonally sensitive tissues and the expression of hormonally sensitive parameters in DART studies. Doses administered in at least some of these studies reached the generally accepted limit dose of 1,000 mg/kg/day. Overall, these data indicate that chloroxylenol is too weak to interact at the estrogen or androgen receptor to induce hormonal effects in the intact animal.

In its review of the available safety data for chloroxylenol (U.S. FDA 2013b), FDA did not identify any studies that addressed the potential hormonal effects of chloroxylenol and concluded that the available data were inadequate to assess potential safety. Review of the literature identified several relevant studies that should be considered by FDA in assessing the hormonal potential of chloroxylenol. These studies are summarized in Tables 2–5, and for the reader's convenience, they are listed in Table 1 above. Taken together, these studies provide an adequate basis to draw conclusions regarding hormonal effects. Further, they indicate that chloroxylenol is unlikely to affect hormonally sensitive endpoints in exposed animals (including humans).

With regard to the above conclusions derived from the *in vivo* mammalian data, a few cautions should be issued. First, for many of the studies detailed in Tables 3 and 4, the original study reports were not available; thus, much of the information presented in the tables is drawn from review articles on chloroxylenol (CIR 1985; Guess and Bruch 1986). Therefore, it is important to access the full record of these studies, and confirm conclusions based on detailed evaluation.

Similarly, details are not available regarding the examination of hormonally sensitive parameters in the studies listed in Table 5 or those discussed in the 2009 EPA registration review preliminary work plan for chloroxylenol (U.S. EPA 2009). Again, it will be important to review the complete studies before forming conclusions. Third, as noted in FDA's draft guidance for assessing the potential hormonal effects of a test substance (U.S. FDA 2013a), it is important to provide a thorough evaluation and to form conclusions based on the full weight of evidence available not only from the *in vitro* studies of receptor activity, but also from the *in vivo* nonclinical safety studies of chloroxylenol.

Overall, the data presented in this report indicate that, although *in silico* and *in vitro* data suggest that chloroxylenol possesses the capability to interact at the estrogen (and possibly the androgen) receptor(s), according to the available *in vivo* mammalian data, its activity is too weak to induce any hormonal effects in an intact organism.

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Appendix D

Dermal and Oral Carcinogenicity and Genotoxicity of Chloroxylenol

Appendix D: Assessment of the Mutagenic and Carcinogenic Effects for Chloroxylenol

Summary of Findings

In the December 17, 2013, Federal Register Notice, FDA is requiring information on the dermal and oral carcinogenic potential of chloroxylenol. FDA identified only one 13-week repeated-dose dermal toxicity study in mice. Literature searches conducted by Exponent identified a dermal carcinogenicity study, additional repeated-dose dermal toxicity studies, oral repeated-dose toxicity studies, and genotoxicity studies conducted with chloroxylenol.

In an 18-month dermal carcinogenicity study in mice, no evidence of carcinogenicity was found. No oral carcinogenicity studies were located in the literature for chloroxylenol. However, based on a weight-of-evidence-based approach, the potential for chloroxylenol to result in oral carcinogenicity is low: oral repeated-dose toxicity studies provided no indication that cancer effects are anticipated, and studies in animals fail to demonstrate genotoxicity. Additionally, because chloroxylenol has been experimentally demonstrated to be absorbed through the skin in rats, the dermal carcinogenicity studies in rodents do indicate that there is no carcinogenic response following systemic exposure to chloroxylenol. Moreover, oral exposure is not anticipated for products containing chloroxylenol; therefore, the dermal carcinogenicity study that has been performed provides the relevant information for a safety evaluation of human uses of chloroxylenol.

Introduction

This section addresses the U.S. Food and Drug Administration's (FDA's) request for more information on the dermal and oral carcinogenicity of chloroxylenol. In order to be responsive to FDA's request for additional information on the carcinogenic effects of chloroxylenol, Exponent performed a detailed literature review to identify appropriate studies for consideration by the FDA in any evaluation of the safety of this chemical for use in the U.S. The literature review included studies on genotoxicity, dermal carcinogenicity, and oral and dermal repeated dose studies. An oral carcinogenicity study with chloroxylenol is not available. To assess the effects of chloroxylenol on oral exposure, a literature review of the available oral repeated dose studies was conducted to ascertain the oral toxicity of these compounds.

Below is information regarding the available studies in these technical areas. We identify the studies that were listed in the Proposed Rule, and then identify and discuss several studies that FDA does not include in their documentation to date, and that should be considered in any safety evaluation for chloroxylenol.

FDA Genotoxicity Requirements

FDA follows the International Conference on Harmonization (ICH) guidelines for genotoxicity (ICH 1997). The guideline for genotoxicity recommends a battery of *in vitro* and *in vivo* studies, including the following:

1. A test for gene mutation in bacteria
2. An *in vitro* test with cytogenetic evaluation of chromosomal damage with mammalian cells or an *in vitro* mouse lymphoma thymidine kinase (tk) assay
3. An *in vivo* test for chromosomal damage using rodent hematopoietic cells.

Chloroxylenol Genotoxicity Database

FDA did not identify any available genotoxicity studies in the December 2013 Federal Register Notice. These studies are included in this document because they are relevant to the mutagenic and carcinogenic assessment of chloroxylenol.

The chloroxylenol genotoxicity studies identified in this effort are shown in Table D1. Overall, the available studies provide a strong database across a diversity of test organisms, in both in vitro and in vivo test systems. Taken together, the available studies indicate that chloroxylenol is not associated with genotoxic effects *in vitro* or *in vivo.*

Table D1. Available chloroxylenol genotoxicity studies

Study Type	Study Detail	Study Result	Reference	Other Identifying Information
Ames Assay (Bacterial Reverse Mutation Assay)	*S. typhimurium* standard strains	Negative with and without metabolic activation	May, 1988 (as cited in EPA RED 1994, HHRA 2009)	MRID 41310301
Ames Assay	*S. typhimurium* (TA 1535, TA 1537, TA 1538, TA 1978, TA 98, TA 100) at 0.2-1.0 µg/plate	Negative with and without metabolic activation	Erco Energy Resources Company, as cited in Cosmetic Ingredient Review, 1985	
Unscheduled *In Vitro* DNA Synthesis	Primary rat hepatocytes; up to cytotoxic levels	Negative	EPA RED 1994, HHRA 2009	MRID 40704101
In Vitro Chromosome Aberration Test		Equivocal - significant but non-dose related increases in the frequencies per cell at two of the highest doses for Chinese Hamster Ovary cells	EPA HHRA 2009	MRID 40704102
In Vivo Mouse Micronucleus Assay		Negative	Ivett , 1989 (as cited in EPA RED 1194, HHRA 2009)	MRID 41085301

FDA Carcinogenicity Requirements

FDA follows the ICH guidelines for carcinogenic effects (ICH 1995). The guideline for carcinogenicity recommends that carcinogenicity studies be conducted for products that are intended to be used continually for 6 months by a route of exposure that is the same as the intended clinical route for exposure. As stated in the December 17, 2013, Federal Register Notice, FDA is also requesting an oral carcinogenicity study: "Because of potential systemic exposure, an oral carcinogenicity study is also necessary to characterize the systemic effects from long-term exposure."

Chloroxylenol Carcinogenicity Database

In the December 17, 2013 Federal Register Notice, FDA did not identify any available oral or dermal carcinogenicity studies for chloroxylenol. FDA did cite a 13-week dermal repeated-dose toxicity study in mice available in Docket No. 1975N-0783H. No oral repeated-dose toxicity studies were cited by the FDA.

The literature review for chloroxylenol indicated that an 18-month dermal combined chronic toxicity and carcinogenicity study in mice is available for chloroxylenol. In addition, the 13-week dermal toxicity study cited by the FDA was reviewed. Additional oral and dermal repeated-dose studies were identified for chloroxylenol that were not cited by the FDA.

The available studies provide evidence that chloroxylenol is not carcinogenic in mice via the dermal route of entry, the intended clinical route of exposure (Momma et al. 1988). Similarly, a variety of studies in several test organisms, with both in vitro and in vivo test systems have demonstrated no evidence that chloroxylenol is genotoxic. Although an oral carcinogenicity study is not available in the literature for chloroxylenol, oral repeated-dose studies in dogs and rats indicate very low systemic toxicity (NOAEL of 1250 mg/kg bw/day) associated with oral exposure to chloroxylenol. Across these studies, there have been no findings that suggest pre-cancerous effects following oral exposure.

EPA Conclusions

In the 1994 Reregistration Eligibility Decision (RED), EPA concluded that the toxicological database on chloroxylenol was adequate and supported reregistration eligibility. U.S. EPA (1994) also states that, except for eye irritation, no toxicological endpoints of concern for acute, short term or chronic exposure to chloroxylenol through occupational or residential exposure have been identified. U.S. EPA (1994) found that the body of data available on chloroxylenol was sufficient to allow the Agency to assess the registered uses of chloroxylenol and to determine that chloroxylenol can be used without resulting in unreasonable adverse effects to humans and the environment.

The EPA Summary of Human Health Effects Data for Chloroxylenol (2009) indicates a NOAEL for repeated oral dose toxicity of 1250 mg/kg bw/day based on the 90-day oral toxicity study in rats. U.S. EPA (2009) also states that, if risks are identified in the Tier I dietary

assessment, chronic toxicity and carcinogenicity studies may be needed. U.S. EPA (2009) is requesting a reiteration of the *in vitro* mammalian chromosome aberration test with chloroxylenol, as well as a 90-day inhalation study, to assess occupational and residential exposures. In the 2009 Chloroxylenol Summary Document, EPA states that structure/activity relationships (SAR) assessments and quantitative (Q)SAR modeling "are another set of tools that are available to Agency scientists. The EPA's Office of Pesticide Programs has begun a process shift that envisions shifting from the current study-by-study approach to an approach in which the use of predicted data, generated using validated models, is considered, along with information from open literature and studies specifically generated under Part 161 requirements," and, "All relevant information would be considered as part of a weight-of-the-evidence evaluation. If stakeholders believe that submission of predicted data can fulfill one of the data needs for chloroxylenol, then the Agency invites submission of this information."

Chloroxylenol Dermal Chronic and Carcinogenicity Study—Mouse

Repeated-dose dermal toxicity studies with chloroxylenol have been identified in the literature. The database of available repeated-dose dermal toxicity studies does not indicate any systemic toxicity at exposures of up to 1000 mg/kg bw/day; local dermal irritation was reported starting at 180 mg/kg bw/day. One study on the chronic toxicity and carcinogenicity from dermal application of chloroxylenol has been identified. This large-scale study used high doses of chloroxylenol applied to the backs of mice for a period of 18 months. From this study, the authors concluded that there was no treatment-related chronic toxicity in any dose group, and that there was no significant difference between the control group and the treatment group in the carcinogenicity study. These studies are included in Table D2 below, and brief descriptions of these studies are provided in this section.

Table D2. Available chloroxylenol dermal repeated-dose and carcinogenicity studies

Study Type	Study Details	Reference	Other Identifying Information
Dermal toxicity study in rabbits (21 days and 90 days)	Albino rabbits; Dosage levels of 0, 18 and 180 mg/kg bw/day	Doyle and Elsea, 1965	MRID 40223124
Dermal toxicity study in rabbits (28 day)	Albino rabbits (5/sex/dose); Dosage levels of 0 or 2,000 mg/kg bw/day of 0.25% chloroxylenol	Mastri and Kepling, 1973	
Dermal toxicity study in mice (49 days)	Crl:CD® -1 (ICR)BR mice (5/sex/dose); Dosage levels of 0.2%, 0.4%, 0.8% or 1.6% in acetone. The dosages were increased weekly till consistent dermal irritation was observed at dosages of 19.2%, 25.6%, 38.4% and 51.2% during week 5 of the study period.	EPA HHRA 2009	MRID 46030401
13-week dermal repeated dose toxicity study in mice	Cr l:CD®-1 (ICR)BR mice (10/sex/dose); Dosage levels 0, 250, 500, and 1000 mg/kg bw/day	Morris, 2001	MRID 46092002; FDA Docket No. 75N-0183
18 month combined dermal chronic toxicity and carcinogenicity study in mice	Slc/ddY specific pathogen-free (SPF) female mice (50-70/dose for carcinogenicity; 10/dose for chronic toxicity); Dosage levels 0, 1% or 10%. Not carcinogenic.	Momma et al., 1988	

In the study conducted by Mastri and Kepling (1973), 0.25% chloroxylenol was applied to albino rabbits (five males and females per dose group) at concentrations of 0 or 2,000 mg/kg bw/day for 28-days. No effect was reported on mortality, clinical signs, body weight, skin irritation, hematological or blood chemistry studies, urine analysis, or gross pathology. Histopathology revealed dermal irritation related findings.

The 2009 EPA Human Health Risk Assessment (HHRA) includes a subchronic dermal toxicity study (MRID 40223124), which was conducted with albino rabbits (Doyle and Elsea 1965). The rabbits (male and female, total of nine animals per dose group) were treated in two groups. One group of rabbits was treated for 21 days, and the other group was treated for 90 days. The dose levels were 0, 18, and 180 mg/kg bw/day chloroxylenol. EPA identified the systemic no-observed-adverse-effect level (NOAEL) as 180 mg/kg bw/day and the NOAEL for skin irritation as 18 mg/kg bw/day based on erythema, coriacious area, and fissuring at a lowest-observed-adverse-effect level (LOAEL) of 180 mg/kg bw/day.

The 2009 EPA HHRA also includes a supplemental range-finding study with chloroxylenol (MRID 46030401). In this study, chloroxylenol was administered dermally to the shaved skin to 5 Crl:CD® -1 (ICR)BR mice for 49 consecutive days at initial dosage concentrations of 0.2%, 0.4%, 0.8%, or 1.6% in acetone. The dosages were increased weekly until consistent dermal irritation was observed at dosages of 19.2%, 25.6%, 38.4%, and 51.2% during week 5 of the study period. EPA identified the LOAEL as 19.2% (213 mg/kg bw/day in males and 284 mg/kg bw/day in females) based on very slight edema, erythema, and desquamation, and stated that a NOAEL could not be established from this study. Gross necroscopy findings for the treated skin in all of the chloroxylenol-treated groups consisted of desquamation, cracking, and/or thickening. Hypergranulosis, hyperkeratosis, epithelial hyperplasia, and acute inflammation within the superficial dermis were noted for almost all of the treated animals during microscopic examination. There was no apparent dose-response relationship in either the incidence or severity of these findings. A systemic NOAEL and LOAEL could not be established due to the lack of adverse treatment-related effects. No systemic effects were observed up to the highest dose of 51.2%.

The Morris (2001) subchronic dermal study was conducted with 10 Cr l:CD®-1 (ICR)BR mice at dosage concentrations of 0, 15%, 30%, and 60% chloroxylenol in 10 µL acetone (0, 250, 500, and 1000 mg/kg bw/day). Macroscopic treatment-related effects included dermal irritation of very slight erythema and edema in ≥250 mg/kg bw/day dose level and thickening and scabbing of the skin at the 1,000-mg/kg bw/day dose level. EPA stated that the LOAEL was established at 250 mg/kg bw/day, the lowest dose tested. A NOAEL could not be established. Based on the study, a systemic LOAEL of 1000 mg/kg bw/day was established. Target organ effects included granulocytic hyperplasia of the bone marrow and lymphocytic hyperplasia of the mesenteric lymph nodes. The NOAEL could not be established, because no organ histopathology was established at the other dose levels.

A dermal combined chronic toxicity and carcinogenicity study of chloroxylenol (parachlorometaxylenol, PCMX) was conducted in 5-week-old Slc/ddY specific pathogen-free (SPF) female mice (Momma et al. 1988). In the carcinogenicity evaluation included in this study, PCMX was applied at concentrations of 0 (control-olive oil, 70 animals), 1%, and 10% (50 animals per dose group) PCMX dissolved in ethanol, twice weekly for 18 months. An

additional 10 animals were allocated to the control, 1%, and 10% treatment groups, respectively, as satellite groups and were used for the concurrent chronic toxicity study (five animals per dose group sacrificed at 6 and 12 months). Animals were observed for clinical signs and mortality twice daily. Animals found dead or moribund were dissected immediately, and postmortem findings were recorded. Body weight and food consumption were measured for each cage, once weekly until week 27, and biweekly thereafter.

For hematological analyses, blood samples were collected from 20 randomly selected control animals, and 10 animals each from the treatment groups, under non-fasted conditions at the time of sacrifice. Serochemistry analysis was performed on the animals at necropsy in the chronic toxicity study (6 and 12 months). At the end of the treatment period, all animals that survived were sacrificed by exsanguination. After autopsy findings were recorded; absolute organ weights of the brain, heart, lung, liver, kidney and the spleen were recorded; and relative organ weights were calculated. Microscopic examination was performed on samples of the brain, heart, lung, liver, kidney, spleen, ovaries, pituitary, thyroid gland, submandibular gland, thymus, adrenals, esophagus, stomach, pancreas, duodenum, jejunum, ileum, mesenteric lymph nodes, uterus, spinal cord, femur, and bladder.

In the chronic toxicity portion of the study, no significant differences in mean body weight and food consumption were reported between groups. No abnormal clinical findings were reported. Only one animal in the 1% treatment group died during Week 45, and tumor of the subcutaneous tissue and fading of the liver and kidney were reported in this animal. No significant difference was reported in hematological findings between the control group and the PCMX treatment groups at the 6^{th} and the 12^{th} month. A significant decrease of free fatty acid, neutral fat, total cholesterol, and phospholipid was reported in the 1% treatment group compared with the control group at 6months, whereas no changes were reported in the 10% treatment group during the same period. A significant decrease of free fatty acid and a significant increase of serum alkaline phosphatase (exhibited dose-response relationship) were reported in the 10% treatment group at 12 months. No significant difference was reported among the groups in the absolute weight and relative weight of each organ both at the 6^{th} and 12^{th} months. No significant findings were reported at necropsy in all three groups at the 6^{th} and 12^{th} month.

In the carcinogenicity portion of the study, no clinical findings or mortality were attributed to treatment. No significant changes in mean body weight and mean food consumption were reported between control and treated groups at all dose levels. A significant increase in red blood cell (RBC), hemoglobin (Hgb), and mean corpuscular hemoglobin concentration (MCHC) was reported in the 1% treatment group, and in Hgb and MCHC in the 10% treatment group compared with the control group. No significant difference was reported in differential leucocyte counts between treatment and control groups. The increase in RBC, Hgb, and MCHC was considered by the authors to be slight when compared to background data, and therefore not abnormal. There was a significant decrease in absolute weight of the spleen in the 1% and 10% treatment groups. There was a significant decrease in the absolute and relative weights of the liver in the 10% treatment group. There were no treatment-related histopathological findings in all animals (those that died during the study and those that survived). There were no treatment-related tumors in the animals that died during the study or in the animals that survived and completed the study (18 months).

The authors concluded that, in the chronic toxicity study, "there were no findings that are considered to be treatment-related." The authors also conclude that there was no increase in carcinogenicity in mice on dermal application of chloroxylenol in this study.

Chloroxylenol Oral Carcinogenicity

In the December 17, 2013 Federal Register Notice, FDA did not identify oral carcinogenicity studies for chloroxylenol.

No oral carcinogenicity study with chloroxylenol has been identified. Because the anticipated route of human exposure to chloroxylenol is via dermal contact, the focus to date has been to evaluate carcinogenicity via this relevant exposure pathway, which is in accordance with the requirements of FDA's guidelines for carcinogenicity testing (ICH 1995). However, in the December 17, 2013 Federal Register Notice, FDA stated that "because of potential systemic exposure, an oral carcinogenicity study is also necessary to characterize the systemic effects from long-term exposure." To assess the effects of chloroxylenol on oral exposure, literature reviews of the available oral repeated-dose studies were conducted to ascertain the toxicity of these compounds on oral administration.

In Table D5 of the December 17, 2013 Federal Register Notice, FDA states that carcinogenicity studies are used to identify "the systemic and dermal risks associated with drug active ingredients." Taken together, these studies are used to identify the type of toxicity, the level of exposure that produces this toxicity, and the highest level of exposure at which no adverse effects occur, referred to as the ''no-observed-adverse-effect level'' (NOAEL). The NOAEL is used to determine a safety margin for human exposure. This information can be extrapolated from the available oral repeated-dose studies, which indicate that the 90-day oral NOAEL for systemic toxicity for chloroxylenol is 1250 mg/kg bw/day in rats. Therefore, based on the available data for chloroxylenol, a NOAEL to determine a safety margin for human exposure exists.

Oral carcinogenicity studies conducted with chloroxylenol have not been identified in the literature. However, there are oral repeated-dose studies conducted with chloroxylenol, and these studies are presented in Table D3. The oral repeated-dose studies available with chloroxylenol indicate that chloroxylenol does not induce adverse effect on any endpoints evaluated. Although some effects have been observed following oral dosing, these effects (e.g., increased absolute and relative liver weights observed following oral administration of chloroxylenol for 13 weeks to rats and beagle dogs) are not considered to be adverse and are considered to be adaptive, because they were not accompanied by biochemical or histopathological changes indicative of liver toxicity. Although negative for toxicity for non-cancer measurement endpoints, these studies were reviewed for insights they might provide with regard to the potential for carcinogenicity from oral dosing of chloroxylenol.

Table D3. Available chloroxylenol oral repeated-dose studies

Study Type	Study Details	Reference	Other Identifying Information
4-week oral gavage toxicity in rat	Specific pathogen free rats (strain unspecified) (5/sex/dose); Dosage levels equivalent to 0, 60, 120 or240 mg/kg bw/day	Hunter et al., 1973a, Huntingdon Research Center (as cited in Guess and Bruch, 1986).	FDA Docket No. 75N-0183
13-week oral gavage toxicity in rat	Sprague-Dawley rats, CFY strain (15/sex/dose); Dosage levels: estimated to be 0, 1.1, 55 and 110 mg/kg bw/day[1]	Hunter et al., 1973b, Huntingdon Research Center.	MRID 00141330; FDA Docket No. 75N-0183
13-week oral gavage study	Rats (strain unspecified). Dosage levels: 8, 24 and 75 mg/kg bw/day.	Harr for Pennwalt company, 1978 (as cited in Enviro-systems)[2].	
4-8 week oral gavage toxicity in beagle dogs (initial study)	Beagle dogs (1/sex/dose); Dosage levels: estimated to be 96, 192, 120 and 384 mg/kg bw/day[3]	Chesterman et al., 1973a, Huntingdon Research Center.	FDA Docket No. 75N-0183
13-week oral gavage toxicity in beagle dogs	Beagle dogs (3/sex/dose); Dosage levels: estimated to be 0, 1.2, 60 and 120 mg/kg bw/day[4]	Chesterman et al., 1973b, Huntingdon Research Center.	FDA Docket No. 75N-0183

A 4-week oral toxicity study of chloroxylenol (as Dettol—a product containing 4.8% chloroxylenol, 10% alcohol, and 20% terpinol in a castor-oil soap base) was conducted to gain insight into the potential toxicity of Dettol as a prelude to a 13-week study (Hunter et al. 1973a, as cited in Guess and Bruch 1986). Male and female specific pathogen-free (SPF) rats (five animals per sex, per dose group) were administered Dettol by oral gavage daily for 4 weeks. The Dettol was diluted to 25% and 50% solution or used at 100%, corresponding to doses of 60, 120, or 240 mg chloroxylenol/kg bw-day, with a constant volume of 5 mL/kg. Control animals received the vehicle alone at the same dose volume. Salivation and increased resistance to handling were reported at 240 mg/kg bw/day. Decreased body-weight gain and reduced food intake were reported in female rats. Similar results were also reported for rats in the 120-mg/kg bw/day dose group. Significant increases in absolute and relative kidney weights was reported in male rats, but the increases were not dose related. A statistically significant increase in liver weights of male rats was reported. Histopathological and clinical pathology results were not reported by Guess and Bruch (1986).

[1] These doses are estimated by Exponent based on the dose of 110 mg/kg bw/day for 5 mL/kg of 50% Dettol dilution provided by Guess and Bruch (1986).

[2] http://envirosi.com/chloroxylenol-toxicology/

[3] The dose levels of 192 and 120 mg/kg bw/day were estimated by Exponent based on the doses of 96 mg chloroxylenol/kg bw/day for the low dose and 384 mg chloroxylenol/kg/day for the high dose (8 mL/kg/day) provided by Guess and Bruch (1986).

[4] Guess and Bruch (1986)

In a subchronic 90-day oral toxicity study (Hunter et al. 1973b), Sprague-Dawley rats, CFY strain (15/sex/dose) were orally administered Dettol (contains 4.8% chloroxylenol, 10% alcohol, and 20% terpinol in a castor-oil soap base) as an emulsion in water, daily for 13 weeks in the following dosing regimen: 0, 0.5 mL/kg/day of a 5% emulsion, 5.0 mL/kg /day of a 25% emulsion, or 5.0 mL/kg/ day of a 50%-emulsion. These are estimated to be equivalent to 0, 1.1, 55, and 110 mg/kg bw/day, based on the equivalent dose of 110 mg/kg bw/day for 5 mL/kg of a 50% Dettol dilution (Guess and Bruch 1986). No deaths were attributed to Dettol administration during the 13-week study. Greater urine volume and higher water intake were reported in males of the high-dose group than in the control animals. In high-dose males, decreased packed cell volume and hemoglobin, and increased total leukocyte and lymphocyte counts were reported. Absolute and relative liver weights of all treated males and high-dose females were significantly higher than controls. In addition, relative kidney weights were significantly higher in mid-dose and high-dose males. No macroscopic or histopathological changes were seen in any treated animals that could be attributed to Dettol administration, and no treatment-related blood biochemical changes were reported.

In a subchronic 90-day oral gavage study in rats by Harr reported for Pennwalt Company in 1978 (as cited in Envirosystems), PCMX in propylene glycol (3.75% PCMX in 50% propylene glycol) was administered to rats daily for 90 days at doses of 8, 24, and 75 mg/kg bw/day. There were no treatment-related effects on body weight, pathology, clinical signs, and hematology assessments at the 8-mg/kg bw/day dose level. At both the 24- and 75-mg/kg bw/day doses, the effects were mild, including slight hemoconcentration, leukocytosis, and monocytosis, with no dose/effect relationship. At the highest dose, the animal responses were considered nonspecific, with some animals showing nasal and ocular exudates. The authors did not identify specific target organs.

In a 4- to 8-week oral toxicity study (Chesterman et al. 1973a), pure-bred beagle dogs (one/sex/dose) were administered Dettol (contains 4.8% chloroxylenol, 10% alcohol, and 20% terpinol in a castor-oil soap base) by gavage in the following dosing regimen: 2 mL/kg/ day of undiluted solution for 4 weeks; 4 mL/kg/ day of undiluted solution for 4 weeks, followed by 5 mL/kg /day of a 50% solution for 4 weeks; and 8 mL/kg/day of undiluted solution "for up to 3½ weeks." The doses of chloroxylenol administered were calculated by Guess and Bruch (1986) to range from 96 mg/kg bw/day at the low dose to 384 mg/kg/day for the high dose. The 4-mL/kg/day undiluted is therefore equivalent to approximately 192 mg/kg bw/day, and the 5-mL/kg/day of a 50% dilution is equivalent to approximately 120 mg/kg bw/day (estimated by Exponent). No control group was indicated. There were no significant signs of toxicity (except vomiting) from either the 2- or the 4-mL/kg/day dose administered for 4 weeks and followed by a dose of 4 mL/kg/day of the 50% dilution for 4 additional weeks. At the high dose level (8 mL/kg/day of undiluted solution), diarrhea, hind limb weakness, ataxia, and a steady weight loss were reported. Gross macroscopic abnormalities were reported at this high dose. Edema of the pancreas and congestion of the kidneys were reported in one dog. A decreased thymus weight was reported in both sexes compared to control. Decrease in splenic and pancreatic weights of the high-dose male dog compared to normal was reported. The authors concluded that 5 mL/kg/day of a 50% solution of Dettol would be a suitable high dose level for a long-term study. According to Guess and Bruch, 5 mL/kg/day of a 50% solution is equivalent to 120 mg chloroxylenol/kg bw/day.

In a subchronic oral toxicity study (Chesterman et al. 1973b), beagle dogs (three/sex/dose) were orally administered Dettol (contains 4.8% chloroxylenol, 10% alcohol, and 20% terpinol in a castor-oil soapbase) in solution daily for 13 weeks at the following dosing regimen: 0, 0.5 mL/kg/d of a 5% solution; 5 mL/kg/day of a 25% solution, and 5 mL/kg/day of a 50% solution. These were equivalent to 0, 1.2, 60, or 120 mg/kg bw/day chloroxylenol, respectively (Guess and Bruch 1986). No deaths were reported during the study. However, occasional vomiting was reported at higher dose levels (60 and 120 mg/kg bw/ day groups). No adverse effects were noted with respect to body weight, water consumption, or food consumption. No ocular changes and no hematological, biochemical, or histopathologic changes attributable to treatment were reported. Urine analysis at 4, 8, and 12 weeks showed a positive reaction for total reducing substances for all animals receiving 60 or 120 mg/kg bw/day chloroxylenol, which according to the authors, was due to the presence of a metabolite. No gross lesions were reported at necropsy. Absolute and relative liver weights for all dosed groups were significantly higher than the control value. The authors did not identify specific target organs.

As discussed above, no studies have been identified that were specifically designed to address the potential for chloroxylenol to cause cancer following oral exposure. However, some information regarding the potential for cancer from this chemical is suggested from the available studies. Several studies in rats and dogs have been performed over dosing regimens that extend up to 13 weeks. In these studies, the consistent finding is that there is very low systemic toxicity associated with oral exposure to chloroxylenol. Across these studies, there have been no findings that suggest pre-cancerous effects. Additionally, chloroxylenol is absorbed through the skin in rats; as stated in the December 17, 2013 Federal Register notice by FDA, "approximately 65 percent of the applied dose was absorbed at 24 hours after 14 and 28 days of daily dosing." Therefore, dermal carcinogenicity studies in rodents do provide a systemic exposure to chloroxylenol. The dermal exposure study, described above, supports the low potential for cancer following long-term oral exposure. As further support for the lack of carcinogenic effects via oral exposure, chloroxylenol has not been demonstrated to be genotoxic.

Conclusions regarding the Mutagenicity and Carcinogenicity Database

- Chloroxylenol has been demonstrated to not be carcinogenic in an 18-month dermal carcinogenicity study in mice.
- No oral carcinogenicity studies were located in the literature for chloroxylenol.
- Using a weight-of-evidence approach, the potential for chloroxylenol to result in oral carcinogenicity is low. This is based on the results of the dermal carcinogenicity study, available oral repeated-dose toxicity studies, as well as the lack of demonstrated genotoxicity. As stated above, chloroxylenol has been experimentally demonstrated to be absorbed through the skin in rats: "Approximately 65 percent of the applied dose was absorbed at 24 hours after 14 and 28 days of daily dosing" (U.S. FDA 2013). Therefore, dermal carcinogenicity studies in rodents do provide an assessment of cancer risks from systemic exposure to chloroxylenol.

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Appendix E

Absorption Distribution, Metabolism and Elimination

Appendix E: Assessment of the Pharmacokinetics (ADME) Database for Chloroxylenol

Summary of Findings

This technical memo responds to the request for additional information on the absorption, distribution, metabolism, and excretion (ADME) of chloroxylenol by the U.S. Food and Drug Administration (FDA) in the recent Federal Register Notice Proposed Rule to amend the tentative final monograph on the safety and effectiveness of consumer antiseptics (U.S. FDA 2013). Because chloroxylenol has been used safely in a variety of dermally-applied consumer products, few animal or human studies have been conducted to evaluate the ADME of chloroxylenol in humans or animals. Available ADME studies indicate that chloroxylenol can be absorbed through the skin, but it is metabolized and excreted rapidly, and blood levels cannot be detected unless high doses are administered. Some studies were identified that were not discussed or evaluated by FDA, and that could enhance the understanding of the ADME of chloroxylenol.

ADME Studies Identified by EPA

In Table 5 of the Proposed Rule, FDA stated that more oral and dermal ADME studies are needed "to identify toxic systemic exposure levels that can then be correlated to potential human exposure via dermal pharmacokinetic study findings." FDA also stated that dermal studies using multiple formulations under maximum use conditions are needed to "to relate the potential human exposure to toxic drug levels identified in animal studies." In Table 5 of the Proposed Rule, FDA cited two guidance documents for conducting preclinical ADME studies (ICH 2000; U.S. FDA 1997). FDA also cited a guidance document to determine how much of the active ingredient penetrates the skin in humans (U.S. FDA 2005).

U.S. FDA (2013) briefly reviewed two human dermal absorption studies (Jordan et al. 1973a,b) and two animal dermal absorption/metabolism studies (Havler and Rance 1974; Sved et al. 2000). Table E1 lists the studies currently included in the Proposed Rule as providing ADME information for chloroxylenol. Because FDA indicates awareness of these studies, no additional discussion of them is provided in this report.

Table E1. Studies listed by U.S. FDA in the Proposed Rule as relevant to assessing ADME

Authors	Date	Title	FDA Citation
Jordan, BJ, JD Nichols, and MJ Rance	1973	Dettol Bathing Product – Preliminary Volunteer Study	FDA Docket 1975n-0183H
Jordan, BJ, et al.	1973	Human Volunteer Studies on Dettol Bathing Product	FDA Docket 1975n-0183H
Havler, ME and MJ Rance	1977	The Metabolism of p-Chloro-m-Xylenol (PCMX) in Sprauge Dawley and Gunn Wistar Rats	FDA Docket 1975n-0183H
Sved, DW	2000	A Dermal Absorption Study with [14C]-Labeled PCMX in Mice	FDA Docket 1975n-0183H

According to U.S. FDA (2013), the dermal absorption of chloroxylenol has been studied in four human subjects following single and repeated bathing (10 minutes daily for 1 to 10 days) and following a single 30-minute percutaneous application to the back of one subject (Jordan et al. 1973a,b). These studies are unpublished and were not available for review. According to a review by EnviroSystems (2014), Jordan et al. (1973a) added 25 mL of Dettol to 125 L of water (total chloroxylenol of about 120 mg), and four volunteers bathed for 10 minutes daily for 1 to10 days. The peak urine concentration of metabolite occurred between 1 and 4 hours after exposure, and the calculated total amount absorbed and excreted in the urine was about 0.5%. In Jordan et al. (1973b), a single volunteer had 1 mL (46 mg chloroxylenol) of Dettol applied to the back and occluded with foil for 30 minutes. Analyses showed that 15.7% of the dose was on or in the skin after exposure. Analysis of the urine for up to 48 hours showed a recovery of 4.4% chloroxylenol as its conjugate, indicating that about 10% of the applied dose had actually been absorbed (EnviroSystems 2014). The FDA's review of these studies (2013) states that the studies have produced inconsistent results.

The Federal Register cites two rodent studies as providing data related to ADME, one study with mice (Sved et al. 2000) and one with rats (Havler and Rance 1977). According to U.S. FDA (2013), in the study with mice, increasing doses of ^{14}C-labeled chloroxylenol were applied to shaved backs as a single or repeated dose (once daily for 14 or 28 days). Absorption was apparent at all time points and increased with increasing length of exposure. Approximately 50% was absorbed at 24 hours after a single dose, and approximately 65% at 24 hours after 14 and 28 days of daily dosing. The plasma half-life for chloroxylenol was 18, 22, and 12 hours for low-, mid-, and high-dose males, respectively, and 70, 9, and 12 hours for low- to high-dose females, respectively. Tissue concentrations were highest in kidney> liver> brain, and increased between Days 1 and 14. The concentration of chloroxylenol equivalents in liver, kidney, and brain typically increased slightly between 1 and 14 days of dosing, but typically did not increase further through 28 days of dosing, indicating that these tissues are not sites of deposition (Sved 2000). Although not noted by FDA, the concentration of chloroxylenol equivalents in the tongue increased through 1, 14, and 28 days of dosing, suggesting continued oral ingestion of the dermally applied dose. This suggests that oral ingestion occurred through self-grooming, so although the application was to the skin of animals, the absorbed dose would

reflect both percutaneous and oral exposures. Failure to account for the oral exposures would result in erroneously high estimates of percutaneous absorption in this study.

According to U.S. FDA (2013), in a study with rats, chloroxylenol was found in the following tissues: kidney, lung, liver, adrenal glands, skin, heart, ovary, ovarian fat, skeletal muscle, skull, spinal cord, spleen, eyes, femur, and brain (Havler and Rance 1977). Tissue concentrations increased with repeated dosing, up to 1.8-fold in the kidney, up to 3.8-fold in the liver, and up to 8.9-fold in the brain (Havler and Rance 1977). Unlike the concentrations in the liver and kidney, chloroxylenol levels in the brain did not appear to reach steady-state concentrations after 28 days of dosing, particularly at the lower chloroxylenol concentrations. Havler and Rance (1977) identified a minor metabolite of chloroxylenol, hydroxylated chloroxylenol, which represented 10 to 15 percent of the metabolites found in urine. Both chloroxylenol and the minor metabolite are excreted as a mixture of glucuronide and sulfate conjugates. Excretion is largely complete 24 hours after a single dermal application. U.S. FDA (2013) states that "the relevance of these findings from a chronic use perspective cannot be evaluated…"

After reviewing these studies, U.S. FDA (2013) concluded that these studies are inadequate to fully characterize the extent of systemic absorption after repeated topical use or to demonstrate the effect of formulation on dermal absorption. FDA stated further that the administrative record for chloroxylenol still lacks data to characterize the similarities and differences between animal and human metabolism of chloroxylenol under maximal use conditions, and data to help establish the relevance of findings observed in animal toxicity studies to humans. As a result, FDA is requesting the following ADME data for choroxylenol:

1. Human pharmacokinetic studies under maximal use conditions when applied topically that include documentation of validation of the methods used to measure chloroxylenol and its metabolites
2. Animal ADME at toxic exposure levels
3. Data to help define the effect of formulation on dermal absorption.

Chloroxylenol ADME Database

A literature search and review was conducted to identify additional ADME studies conducted in humans or animals that may provide information to address FDA's additional data requests. Very few studies have been conducted to evaluate the absorption, distribution, metabolism, and/or the excretion of chloroxylenol in humans or animals. Our review of the literature identified additional studies, the availability of which was not acknowledged by FDA, but that may provide useful ADME information on chloroxylenol. The chloroxylenol ADME studies identified in this effort are described briefly in Table E2.

Table E2. Available chloroxylenol ADME studies

Study Type	Reference	Study Findings
Elimination study in rabbits and humans	Zondek B. 1942. The excretion of halogenated phenols and their use in the treatment of urogenital infections. J Urology, 48:747-58. Zondek B, Shapiro B. 1943. Fate of halogenated phenols in the organism. Biomed J. 37:592-595.	Two rabbit studies and one human study to evaluate urinary levels of chloroxylenol. Approximately 16.4% of 1 g dermally applied and 15% of 1 g subcutaneously administered chloroxylenol was eliminated in urine over 5 days in rabbits. In one human subject administered 8 g dermally in alcohol/glycerin, 11% was excreted in 48 hours. In one human subject administered 5 mg intragluteally, 14% was excreted with glucuronic acid and 17% with sulfuric acid at 3 days. In a human subject injected with 5 g intragluteally, approximately 14% of chloroxylenol was excreted combined with glucuronic acid, and 17% as a sulphuric ester. In 5 rats treated with 100-200 mg chloroxylenol, 15% was excreted in feces; 30% in urine during 48 hours. Author stated that dermal administration of an ointment containing 50 g resulted in a blood concentration of 3-10 mg/dL in humans (no other details provided).
Human subjects	Zondek B, Finkelstein M. 1946. Blood concentration of p-chloroxylenol in man following parenteral percutaneous and rectal application. Proc Soc Exp Biol Med, 61:200-2.	Three studies in humans (i.m, oral, rectal, dermal) to evaluate blood levels of chloroxylenol. Dermal results presented below. No chloroxylenol was detected in the blood following the dermal administration of 2 g of p-chloroxylenol in an ethanol/olive oil vehicle in human subjects. After a dose of 5 g, “traces” were found, after 8 g, 1 mg % (1 mg/dL) was found in the blood after 3 hours, and 4 mg % (4 mg/dL) after 24 hours. After a dose of 20 g, 4 mg % (4 mg/dL) was measured within ½ hour, and 1 mg % (1 mg/dL) was present at 72 hours.
In Vitro dermal absorption study in pig skin	Gudipati RM and Stavchansky SA. 1995. Percutaneous absorption of parachlorometaxylenol. Int. J. Pharm. 118:41-45.	Discusses cosolvent effects increasing absorption, and reports on testing in surfactant. Reported 18% of a 5% solution of chloroxylenol was extracted from pig skin and reported a permeability coefficient of 2.97×10^{-4} cm/min in pig skin *in vitro*.
In vitro permeability study epidermis to phenolic compounds	Roberts, MS, RA Anderson, and J Swarbrick. 1977. Permeability of human epidermis to phenolic compounds. J. Pharm. Pharmac. 29:677-583.	Trends in dermal absorption for diverse phenolic compounds. Demonstrates threshold concentration below which absorption is slower.
In vitro permeability study	Vijay, V, EM White, MD Kaminski, JE Riviere, RE Baynes. 2009. Dermal permeation of biocides and aromatic chemical in three generic formulations of metalworking fluids. J. Toxicol and Env. Hlth. Part A 72:832-841.	*In vitro* study that evaluates dermal permeation of different oils, including chloroxylenol, on absorption.

Study Type	Reference	Study Findings
In vitro metabolism study	Thomas RE and Kotchevar AT. 2010. Comparative in vitro metabolism of chloroxylenol, chlorophene, and triclosan with rat, mouse, and human hepatic microsomes. Toxicol. Environ. Chem., 92:9, 1735-1747.	Varying concentrations (50, 100, 150, 200, 300, 400, 500, and 600 mmol/L) of chloroxylenol were incubated with rat, mouse, or human liver microsomes to evaluate metabolites. The chemical structures and amounts of metabolites were the same for all three species.
ADME study in dogs	Dorantes A and Stavchansky S. 1992. Pharmacokinetic and metabolic disposition of p-chloro-m-xylenol (PCMX) in dogs. Pharm Res. May 9(5):677-82.	Five mongrel dogs received single iv and oral doses of 200 and 2000 mg chloroxylenol, respectively. Several pharmacokinetic parameters were evaluated after iv or oral administration, including (e.g., AUC, CL, V_{ss}, $T_{1/2}$, metabolites)
Elimination study in dogs	Unknown reference, cited in EPA RED (1995) and Bruch (1996).	Beagle dogs dosed orally excreted virtually all of the chloroxylenol in their urine within 24 hours. A small amount was present in feces, but essentially none remained in any tissue. The chemical was excreted in conjugated form with little free chloroxylenol. (No other details were provided by EPA.)

The two toxicological studies cited in the Proposed Rule were both 28-day repeat-dose dermal studies that evaluated absorption, plasma half-life, and tissue concentrations in mice and rats (Sved 2000; Havler and Rance 1977). The identification and percentages of excreted metabolites were also measured in rats (Havler and Rance 1977). The two human studies were bathing studies with dilute solutions of chloroxylenol reported to be minimally adsorbed from intact skin (Jordan et al. 1973a,b).

U.S. FDA (2013) discussed only the results from the experiments in rats from Havler and Rance (1977); however, there was also an ADME experiment with dogs included in the study. Because the unpublished study report by Havler and Rance (1977) was not available, much of the information summarized below is from the review article on chloroxylenol (Bruch 1996).

According to Bruch (1996), in the study performed by Havler and Rance (1977), the metabolism of $^{(14C)}$chloroxylenol was examined in two strains of rats after i.m., s.c., and oral administration of Dettol and i.v. administration of chloroxylenol (doses were not provided in this review article by Bruch). According to Bruch (1996), the Gunn-Wistar rats, which are deficient in UDP-glucuronyl-transferase, appeared to metabolize chloroxylenol as rapidly as did the Sprague-Dawley rats. In addition to the metabolic studies, tissue distribution studies on chloroxylenol were performed on a variety of tissues at 15-, 30-, 60-, and 120-min intervals. Bruch (1996) stated that there was a rapid appearance of chloroxylenol in plasma, whole blood, and kidney following i.m. injection of Dettol, and blood levels were essentially 0 at1 hour following i.v. injection of chloroxylenol.

In a second experiment conducted to study the metabolism of chloroxylenol, extensive absorption, tissue distribution, and excretion data were generated after oral and percutaneous administration of $^{(14C)}$chloroxylenol to female rats. Dosing was with a 25% solution of Dettol orally at a rate of 4 mg/kg. For percutaneous absorption studies, a 25% solution of Dettol, 4 mg/kg, was applied to the shaved and abraded backs of the rats and occluded with a dressing.

The occluded pad was removed after 6 h and the skin swabbed with cotton wool soaked in detergent. Both the dressing and cotton swabs were extracted in methanol and analyzed for residual labeled chloroxylenol.

According to Bruch (1996), male beagle dogs were also dosed orally with 25% Dettol in water at a rate of 1 mg/kg. Blood samples were taken up to 24 hours after dosing, and urine and feces were collected for 5 days, after which the animals were sacrificed, and selected tissue analyses were performed. According to Bruch (1996), the results of this study showed that practically all of the oral, labeled chloroxylenol was recovered in the first 24 hours, indicating complete absorption. Peak levels were found in the bloodstream 30 min after oral dosing and 2 h after percutaneous administration. Similar pathways and metabolites were found in the rat and the dog and were identified as a 6:1 ratio of glucuronide and sulfate conjugate. In the percutaneous rat study, approximately half of the administered dose was excreted, with most of the remainder being on the lint dressing. The plasma levels following oral administration reached a peak 30 min after dosing, declining steadily until very little remained at 24 hours. After percutaneous dosing, peak plasma levels occurred 2 hours after dosing, declining steadily up to the 6-hour point, when the dressing was removed. Following removal, there was a rapid plasma decline, with very little chloroxylenol in the plasma by 24 hours post-dosing. In the plasma, the level of free chloroxylenol was essentially undetectable, with all the label being in the form of polar metabolites whether the route of administration was oral or percutaneous.

Bernhard Zondek was a physician who conducted several animal and human studies to evaluate the pharmacokinetics of chloroxylenol in the 1940s. Zondek (1942) summarizes two studies in rabbits in which chloroxylenol was administered subcutaneously and measured in urine, and another study in which chloroxylenol was dermally administered and measured in urine. Zondek (1942) also stated that 150 human subjects were administered 6 g chloroxylenol to evaluate the excretion in humans by bacteriological and chemical methods. According to Zondek, human subjects who were administered between 2.5 and 10 g did not have detectable levels of chloroxylenol in their blood. In another study, Zondek and Shapiro (1943) stated that the dermal administration of 50 g chloroxylenol in an ointment resulted in blood concentrations of 3–10 mg/100 mL in humans (no additional information provided). That dose is much higher than those evaluated in the Jordan et al. (1973) studies cited by FDA. Zondek and Shapiro (1943) also evaluated the metabolism of chloroxylenol and reported that it was excreted primarily as the glucoronide (14%) and sulfuric ester (17%) in a human subject. Even with the large doses of chloroxylenol administered by Zondek in humans, there were apparently no adverse systemic reactions reported. Zondek (1943) also injected 1 g/10 mL olive oil subcutaneously in four rabbits, and approximately 15% was excreted over 4–5 days. Zondek and Finkelstein (1946) conducted three studies in human subjects to evaluate blood levels of chloroxylenol following intramuscular injection of 1–2 g, oral administration of 2–10 g, rectal administration of 2–4 g, or dermal application of 2 g chloroxylenenol in olive oil. No chloroxylenol was detected in the blood following the dermal administration of 2 g of chloroxylenol in an ethanol/olive oil vehicle. After a dose of 8 g, 1 mg/dL was found in the blood after 3 hours, and 4 mg/dL after 24 hours. After a dose of 20 g, 4 mg/dLwas measured within ½ hour, and 1 mg/ dL was present at 72 hours. The authors concluded that chloroxylenol is absorbed through the skin and persists for “considerable periods of time”.

Although the studies conducted by Zondek may provide some potentially useful information on the absorption and metabolism of chloroxylenol, these are relatively old studies with little detail on the test subjects (e.g., age, number of subjects, sex), study design, and the analytical methods, which limits their usefulness for evaluating the ADME of chloroxylenol.

Three *in vitro* dermal penetration studies (Roberts et al. 1977; Gudipati and Stavchansky 1995; Vijay et al. 2009) were identified that were not included in U.S. FDA (2013). These studies may provide useful data to evaluate the dermal absorption of chloroxylenol and how absorption may be affected by different formulations. These studies may potentially provide useful data (e.g., a permeability coefficient) to develop a mathematical model of the dermal absorption of chloroxylenol. These studies are described in more detail in Appendix F, Dermal Absorption.

Two studies were identified that evaluated the metabolism or pharmacokinetics of chloroxylenol that were not mentioned by U.S. FDA (2013). The *in vitro* microsomal metabolism study by Thomas and Kotchevar (2010) provides support for the same metabolic pathways for chloroxylenol in mice, rats, and humans. In this study, four oxidized metabolites of chloroxylenol were found in all three species with one metabolite (4-chloro-3-hydroxymethyl-5-methyl phenol) present as the largest peak in all the three systems. A study by Dorantes and Stavchansky (1992) evaluated the pharmacokinetics of chloroxylenol in 5 mongrel dogs and reported several pharmacokinetic parameters that are listed in Table 5 of the Federal Register Notice (FDA 2013). The parameters evaluated by Dorantes and Stavchansky (1992) included the rate and extent an active ingredient is absorbed into the body (e.g., AUC, Cmax, Tmax), information on the presence of metabolites, and how chloroylenol and its metabolites were excreted (e.g., renal clearance, nonrenal clearance, and fraction of drug excreted in urine). In addition, this study confirms the findings by Zondek (1943) that chlorxylenol is primarily excreted as conjugated species (glucuronides and sulfates). This study provides useful data that may address the request for ADME studies in animals and should be considered by FDA. For example, Table E3 lists some of the ADME data obtained for chloroxylenol in this study with dogs. In addition, the amount of unchanged chloroxylenol excreted in urine after oral or intravenous administration resulted in a mean bioavailability value of 21%, indicating a low absorption. Furthermore, biotransformation studies in urine samples indicated that all of the excreted material was in the form of conjugated species (glucuronides and sulfates).

Table E3. Pharmacokinetic data in dogs (Dorantes and Stavchansky 1992)

Pharmacokinetic Parameter	200 mg IntravenousSingle Dose (mean)	2,000 mg OralSingle Dose(mean)
Volume of distribution (V_{ss})	22.45 L	-
Clearance (Cl)	13.76 L/hr	-
($AUC_{(0,24)}$)	14.14 µg hr/mL	375.4 ng hr/mL
Area under the curve ($AUC_{(0,\infty)}$)	15.02 µg hr/mL	434.9 ng hr/mL
Mean residence time (MRT)	1.69 hr	5.16 hr
Elimination half life ($T_{1/2}$)	1.7 hr	-
Elimination constant (ß)	0.407 hr^{-1}	-
Distribution constant (α)	5.74 hr^{-1}	-

EPA Conclusions regarding ADME In the 1994 Reregistration Eligibility Decision (RED), EPA concluded that the toxicological database on chloroxylenol was adequate and supported reregistration eligibility. U.S. EPA (1994) briefly mentioned the unpublished study with Sprague-Dawley rats and dogs by Havler and Rance (1977), which evaluated the pharmacokinetics of chloroxylenol and was also apparently reviewed by U.S. FDA (2013). According to EPA, the study with a 25% solution of chloroxylenol dosed orally or dermally to Sprague-Dawley rats demonstrated that practically all of it was eliminated in the first 24 hours, mostly in the urine, with small amounts in feces. High concentrations were found in the tissues of the kidney, which indicated excretion in urine. Concentrations in the lungs indicated some elimination in expired air. Beagle dogs dosed orally excreted virtually all of the chloroxylenol in their urine within 24 hours, and a small amount was present in feces, but essentially none remained in any tissue. Chloroxylenol was excreted in conjugated form with little free chloroxylenol. EPA did not provide any additional information or analyses concerning this ADME data. In a 2009 summary of health effects data for the chloroxylenol registration review decision document (U.S. EPA 2009), EPA does not discuss any ADME for this chemical and does not request any additional ADME data or studies.

Conclusions regarding the Chloroxylenol ADME database

- Few animal or human studies have been conducted to evaluate the absorption, distribution, metabolism, and/or excretion of chloroxylenol in humans or animals. However, relevant studies have been identified that were not discussed or evaluated by the FDA, which could enhance the understanding of the ADME of chloroxylenol.

- Up to 15% of an applied dose is absorbed through the skin, with the percentage dependent on the applied concentration, length of exposure, degree of occlusion, and other factors. The data support that absorbed chloroxylenol is metabolized and excreted rapidly, so that blood levels are not detected unless very high doses are administered. Furthermore, no toxicities have been reported in the available animal and human ADME studies.

- U.S. FDA (2013) discussed only the results from the experiments in rats from the Havler and Rance (1977) study; however, according to a review on chloroxylenol by Brunch (1996), an experiment with dogs was also included in that study. Data from that unpublished study will be relevant to FDA's evaluation of ADME for chloroxylenol.

- A few studies that were not included in the Federal Register Notice (U.S.FDA 2013), conducted by Zondek and colleagues in the early 1940s, evaluated blood levels, urinary levels, and metabolites in experimental animals and humans following the dermal administration of relatively high doses of chloroxylenol. These studies provide some limited information about the amount of chloroxylenol that is excreted through the urine, and blood levels in animals and humans. However, these are relatively old

studies with little detail on the test subjects, study design, and the analytical methods, which limits their usefulness for evaluating the ADME of chloroxylenol.

- Three *in vitro* penetration studies (Roberts et al. 1977; Gudipati and Stavchansky 1994; Vijay et al. 2009) that were not included in U.S. FDA (2013) may provide useful data to evaluate the dermal absorption of chloroxylenol and how absorption may be affected by different formulations. These studies are discussed in more detail in Appendix F, Dermal Absorption.

- The study by Dorantes and Stavchansky (1992) provides useful ADME data from dogs that were administered single oral or intravenous doses of chloroxylenol. This study was not included in the Federal Register Notice (U.S. FDA 2013).

- The FDA's proposal to conduct animal ADME studies at "toxic levels" runs counter to FDA guidance and is not scientifically justified, for the following reasons:

  – Such levels are not relevant to typical or maximal use levels of chloroxylenol in various products.

  – According to the FDA (1987) document, "Guideline for the format and content of the human pharmacokinetics and bioavailability section of an application," ADME studies should be conducted to show (by measure of plasma drug levels), the rate of drug absorption and delivery to the systemic circulation and the rate of elimination by metabolic or excretory processes within the recommended clinical dosing range.

  – In the FDA "Bioavailability and Bioequivalence Requirements" guideline, FDA recommends that, "The reference material in such a bioavailability study should be a solution or suspension containing the same quantity of the active drug ingredient or therapeutic moiety as the formulation proposed for marketing" (21 CFR Part 320.25, 2012).

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Appendix F

Dermal Absorption of Chloroxylenol, Including Mixtures

Appendix F: Assessment of the Percutaneous Absorption of Chloroxylenol, and Effects of Formulation

Summary of Findings

The Proposed Rule from U.S. FDA (2013) includes chloroxylenol in its list of "over the counter consumer antiseptic active ingredients with no change in classification in this proposed rule compared to the 1994 TFM" (Table 4 of Proposed Rule). However, with this classification, FDA denotes that additional data are needed for both safety and efficacy. As part of the requested data, the Proposed Rule specifically lists the need for additional studies on dermal administration using multiple formulations under maximal use conditions. Table 5 of the Proposed Rule indicates that these studies are requested to provide information on "how much of the active ingredient penetrates that skin leading to measureable systemic exposure," and that the data will be used to "relate the potential human exposure to toxic drug levels identified in animal studies."

This technical memo responds to the request for information on the dermal absorption of chloroxylenol. Table F1 lists the studies currently included in the Proposed Rule as providing information on the dermal absorption of chloroxylenol. Because the U.S. FDA indicates awareness of these studies, no additional discussion of them is provided. Table F2 provides a compilation of studies not listed in the Proposed Rule, but which contain data regarding the potential for absorption following dermal application of chloroxylenol. A brief discussion of each of these studies is provided, including information regarding the relevance of the study to understanding dermal absorption of chloroxylenol. This memo includes information regarding studies of the dermal absorption of chloroxylenol applied alone, and the available information regarding the effect of mixtures on absorption for this antimicrobial.

Chloroxylenol Database on Dermal Absorption

This review indicates that studies on the dermal absorption of chloroxylenol have been conducted on several species of research animals, and in studies using human volunteers. Taken together, the available data on the dermal absorption of chloroxylenol indicate that it can be absorbed following dermal application of high doses, and that absorption is enhanced by damaged (abraded) skin, high concentrations, extended dosing periods, and occlusive covering.

Many of the studies conducted on high concentrations, high loadings, and/or for long periods of exposure provide results that are not relevant to understanding the potential for human exposure from actual uses of products containing chloroxylenol, and these considerations should be evaluated by FDA in their evaluation.

Some of the newer studies regarding percutaneous absorption and the flux of chemical across the skin may be more useful to FDA in their assessment for handwash and other current applications for chloroxylenol than the earlier studies that express the percent of an applied dose that is absorbed, following application of doses that are not relevant to current uses of the chemical. Of particular relevance are those studies that have evaluated absorption from lower concentrations, and particularly those that consider the effect of formulation on absorption. Information regarding the lag time for absorption through the skin is also an important consideration, given the anticipated short duration of exposure from handwash products.

Review of Studies

Given the long experience with chloroxylenol in the U.S. and other countries, several studies have been conducted, some of which demonstrate the potential for the chemical to be absorbed through the skin. The US FDA's recent review of the safety data available for chloroxylenol (US FDA, 2013) specifically identifies four individual studies as providing information on the dermal absorption of chloroxylenol.

Table F1. Studies listed by U.S. FDA in the Proposed Rule as relevant to assessing dermal absorption

Authors	Date	Title	Notes
Jordan, BJ, JD Nichols, and MJ Rance	1973	Dettol Bathing Product – Preliminary Volunteer Study	FDA Docket 75N-0183H
Jordan, BJ, et al.	1973	Human Volunteer Studies on Dettol Bathing Product	FDA Docket 75N-0183H
Havler, ME and MJ Rance	Undated	The metabolism of p-Chloro-m-Xylenol (PCMX) in Sprauge Dawley and Gunn Wistar Rats	FDA Docket 75N-0183H
Sved, DW	Undated	A dermal absorption study with [14C]-labeled PCMX in mice	FDA Docket 75N-0183H

The U.S. FDA is correct that these studies provide data regarding the dermal absorption of chloroxylenol in both in studies conducted both in laboratory animals and with human volunteers. However the studies listed by U.S FDA are dated, and a detailed search identified several additional resources of information that are available and should be considered by U.S. FDA in the evaluation of dermal absorption of chloroxylenol. These additional studies are summarized in Table F2, followed by discussion of the information provide in each. Review articles that have been published in the peer-reviewed literature or a book chapters are listed at the top of Table F2, followed by studies that have been published in the peer-reviewed literature and unpublished studies.

Table F2. Studies on dermal absorption of chloroxylenol NOT listed by U.S. FDA (2013)

Authors	Date	Title	Notes
Review Articles			
CIR Expert Panel	1985	Final Report on the Safety Assessment of Chloroxylenol	Sometimes cited as Elder 1985, or Anonymous 1985
Guess WL and Bruch MK	1986	A review of available toxicity data on the topical antimicrobial, chloroxylenol	Detailed review published in the peer-reviewed literature
Bruch, MK	1996	Chloroxylenol: An old-new antimicrobial	Updates the prior review and provides a summary of available literature
Peer-Reviewed Publications			
Zondek, B.	1942	The excretion of halogenized phenols and their use in the treatment of urogenital infections	Includes evaluation of chloroxylenol in various preparations
Zondek, B and Finkelstein	1946	Blood concentrations of p-chloro-xylenol in man following parenteral, percutaneous, and rectal application	Builds on earlier studies published in 1942 and 1943
Roberts, MS, RA Anderson, and J Swarbrick	1977	Permeability of human epidermis to phenolic compounds	Trends in dermal absorption for diverse phenolic compounds. Demonstrates threshold concentration below which absorption is slower.
Gudipati, RM, SA Stavchansky	1994	Percutaneous absorption of parachlorometaxylenol	Discusses cosolvent effects increasing absorption, and reports on testing in surfactant.
Vijay, V, EM White, MD Kaminski, JE Riviere, RE Baynes	2009	Dermal Permeation of biocides and aromatic chemicals in three generic formulations of metalworking fluids.	In vitro study that evaluates dermal permeation and the effect of different oils on absorption
Unpublished Studies			
Havler, ME, BJ Jordan, S. Malam, and MJ Rance	1974	Metabolism studies of PCMX. Report No 5369/2, Reckitt and Colman Co.	Submitted to FDA Docket 175N-0183, but is not listed in Proposed Rule
Stavchansky, S.	1985	Report to Dexide, Inc. *Includes information regarding the modeling of uptake]*	Submitted to FDA Docket 175n-0183 but is not listed in Proposed Rule

Note: Findings reflected in this table are limited to results related to dermal absorption.

The three review studies (Elder 1985; Guess and Bruch 1986; and Bruch 1996) each provide a meaningful synthesis of the absorption and toxicity studies available at the time of authorship. The review published by Elder (1985) was developed in response to a Cosmetic Ingredient Review conducted for chloroxylenol that was specifically intended to address the issue of safety for use. Bruch (1996) provides an update on the earlier summary published by Guess and Bruch (1986). Each one of these review articles demonstrates the author's familiarity with the research

conducted to date on chloroxylenol, and each of these should be evaluated by FDA as part of any reassessment for this chemical.

Bernhard Zondek was an early pioneer in assessing the absorption and metabolism of chloroxylenol following administration by different routes of exposure. Zondek (1942) summarizes a study in rabbits and discusses the varying absorption based on the composition of the applied material. In this study, 1 gram of chloroxylenol applied to the skin of rabbits resulted in urinary excretion of 16.4% of the dose administered as an alcoholic tincture. Administration in various ointments resulted in urinary excretion between 7% and 12% of the applied dose.

Zondek and Finkelstein (1946) includes a section describing percutaneous administration with human patients. Dermal application of high concentrations (40%) of chloroxylenol in mixture of alcohol and oil provided early evidence of dermal absorption, and also demonstrated the ability of the skin to reduce the absorption of the administered dose, relative to oral administration. In this study, application of a 2-gram dose of chloroxylenol to the skin of 11 patients did not result in measurable concentrations of the chemical in blood. A 5-gram application resulted in traces in the blood, and an 8-gram application resulted in blood concentrations of 1 mg/dL after 3 hours, and 4 mg/dL after 24 hours. A larger applied dose of 20 grams resulted in 4 mg/dL of chloroxylenol in the blood within half an hour of application, and 1 mg/dL "even after 72 hours." The authors ascribed the long presence of chloroxylenol in blood after dermal application to "the slow absorption… from skin which thus acts as a depot."

Roberts et al. (1977) included chloroxylenol in a study of dermal absorption across a variety of phenolic chemicals. For chloroxylenol, these researchers report a permeability coefficient of 9.84×10^{-4} cm/min (0.059 cm/hr) using an *in vitro* system with separated human abdominal skin. A couple of particularly interesting points from this research pertain to findings on the effect of concentration on absorption, and the discussion of lag time for percutaneous absorption. Although chloroxylenol concentrations tested in this study did not exceed the threshold, these authors discuss how, for many phenolic compounds, there is a concentration threshold above which the flux of the chemical through the skin becomes elevated, presumably due to skin damage at high concentrations. This may be a very important consideration when interpreting most of the available data regarding the dermal absorption of chloroxylenol, because while many of these studies indicate a high percentage of absorption, they are conducted at extremely high concentrations that are not relevant to human use scenarios for handwashing. In fact, the concentration of chloroxylenol in undiluted hand wash falls an order of magnitude below the concentrations tested in many of the studies of percutaneous absorption.

Another important consideration discussed by Roberts et al. (1977) relates to the lag time for the permeability of phenolic compounds through the skin. For chloroxylenol, the reported lag time is 18 minutes. Given the anticipated use of chloroxylenol in antiseptic hand wash, where it is unlikely that the chemical would remain on the skin for more than 30 seconds, FDA should incorporate the consideration of lag time in their assessment of potential exposures and associated safety.

The study by Gudipati and Stavchansky (1995) investigates the effect of surfactant concentration on the percutaneous absorption of chloroxylenol using an *in vitro* system and pig

skin. The authors report that the "total permeability coefficient of chloroxylenol through pig skin was found to be 2.97×10^{-4} cm/min (0.018 cm/hr), and that the fraction retained in the skin (18.1%) was "significant." Additionally, this paper discusses the potential for co-solvents to act as "skin penetration enhancers." This issue may be particularly important in the interpretation of early studies of high loads of chloroxylenol delivered in solvent systems such as DMSO and other organic systems. It is also particularly relevant because of the focus in this research on the effect of dermal application of chloroxylenol in "Tween 80," a surfactant and emulsifier frequently used in soaps and cosmetics. This study, which evaluates the dermal absorption of chloroxylenol at concentrations that are below the solubility limit for consumer products, and that are tested within a relevant formulation, should be considered by FDA in any reevaluation of chloroxylenol.

Although the study by Vijay et al. (2009) aimed to improve understanding of biocides in metalworking fluids (MWF), it contains information that is useful in informing FDA's analysis specified under the Proposed Rule. This evaluation of the dermal absorption of biocides from different types of MWF includes an evaluation of the dermal absorption of chloroxylenol from water and from mixtures containing different oils. Using an *in vitro* research model and pig skin, this study reports on the dermal permeability coefficient of 100 ppm (0.01%)chloroxylenol in water. It also provides data on the impact of mixtures on absorption, including an oil-free synthetic cutting fluid, as well as low-oil and high-oil fluids. Increasing oil content of the MWF was associated with a slight decrease in dermal permeability for fluids containing 100 ppm chloroxylenol. More interestingly, the presence of any MWF resulted in at least a 10-fold decrease in dermal permeability. Specifically, 10.6% of the dose was absorbed from water, vs. 0.81% to 0.41% for MWF of increasing oil content. Permeability coefficients are also reported (as Log K_p values), and the corresponding values are –1.54 for water and -2.63 to –2.92 for increasing oil content. This information is important for FDA to consider for placing historical dermal absorption research on chloroxylenol into the context of current usage in terms of concentration and formulation.

It appears from a review of available information that there are unpublished studies with relevant information that have been submitted to FDA in the past (Docket 75N-0183), but that are not listed in the Proposed Rule. Two of these include a study by Havler et al. (1974) and Stavchansky (1985). The first of these included evaluation of the percutaneous absorption of chloroxylenol following a 6-hour exposure on abraded skin under an occlusive patch. The authors report that approximately half of the administered dose was excreted (Bruch 1996). Due to the study design (e.g., abraded skin, occlusive patch), results from this effort will provide a high estimate of dermal absorption from handwash products.

The unpublished study by Stavchansky (1985) is a computer modeling effort that uses data from two human bathing studies (Jordan 1973a and 1973b, both of which are listed as information sources in the Proposed Rule), to model effects of dosing outside of the conditions studied. This effort indicated that the rapid metabolism of chloroxylenol results in little potential for accumulation (Bruch 1996), even after administration of elevated doses.

Summary

This review indicates that studies on the dermal absorption of chloroxylenol have been conducted on several species of research animals, and in studies using human volunteers. Taken together, the available data on the dermal absorption of chloroxylenol indicate that it can be absorbed following dermal application of high doses, and that absorption is enhanced by damaged (abraded) skin, high concentrations, extended dosing periods, and occlusive covering.

Currently, the evaluations of chloroxylenol that have been conducted by EPA have little reference to the potential for or magnitude of percutaneous absorption. The reregistration eligibility decision (RED) (U.S. EPA 1994) states only, "Following dermal exposure, about half of the material was not absorbed." The analysis does not discuss the relevance of the loading dose, condition of the skin, or even the species on which the conclusion was drawn. A more recent *Summary of Human Health Effects Data* (U.S. EPA 2009) provides a brief review of toxicity studies, but includes no insights for FDA on dermal absorption.

Many of the studies conducted on high concentrations, high loadings, and/or for long periods of exposure provide results that are not relevant to understanding the potential for human exposure from actual uses of products containing chloroxylenol, and these considerations should be evaluated by FDA in their evaluation.

It is important that FDA conduct a more detailed review of information available to them before proceeding with any chemical evaluations. This is particularly important because many of the studies submitted to the docket may not be publicly available.

Some of the newer studies regarding percutaneous absorption and the flux of chemical across the skin may be more useful to FDA in their assessment for hand wash and other current applications for chloroxylenol than the earlier studies that express the percent of an applied dose that is absorbed, following application of doses that are not relevant to current uses of the chemical. Of particular relevance are those studies that have evaluated absorption from lower concentrations, and particularly those that consider the effect of formulation on absorption. Information regarding the lag time for absorption through the skin is also an important consideration, given the anticipated short duration of exposure from handwash products.

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