Dermal Sensitization Assessment for Formaldehyde – Human Studies

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Outline

- Purpose of Presentation
- Dermal sensitization and formaldehyde
- Allergic Contact Dermatitis (ACD)
- Presentation of studies
  - Flyvholm et al. 1997
  - Fischer et al. 1995

Introduction and Purpose

- The Office of Pesticide Programs (OPP) and the Office of Pollution, Prevention, and Toxics (OPPT) are evaluating the risks from exposure to formaldehyde under their respective statutes.

- The Agency is consulting with the HSRB on the scientific and ethical conduct for 2 intentional human exposure studies that examined elicitation thresholds from dermal exposure to formaldehyde.

OPP and OPPT differences

OPP

- Works under Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), which governs the registration, distribution, sale, and use of pesticides in the United States.
- Pesticides are required to undergo periodic re-evaluation to ensure they continue to meet the standard of no unreasonable adverse effects on human health and the environment
- FIFRA registered use sites for formaldehyde include agricultural, food handling, veterinary, commercial/industrial/institutional, water systems, **materials preservation of industrial and household products (Most relevant to dermal exposure)**

OPPT

- Works under the Toxic Substances Control Act (TSCA)
- Provides EPA with authority to require reporting, record-keeping and testing, and restrictions relating to chemical substances and/or mixtures
- TSCA registered use sites for formaldehyde include incorporation into articles, incorporation into a formulation, mixture, or reaction product for various industrial, commercial, and consumer applications including textiles, foam bedding/seating, semiconductors, resins, glues, composite wood products, **paints, coatings, plastics,** rubber, resins, construction materials, **furniture, toys,** and various adhesives and sealants **(Most relevant to dermal exposure)**

Why consider dermal sensitization endpoints for formaldehyde?

- Formaldehyde is a known dermal sensitizer
- In contrast to dermal irritant effects which are reversible, after chemical sensitization is induced in an individual, it may last a lifetime
- Ongoing work within EPA and outside organizations, particularly the Organisation for Economic Co-operation and Development (OECD), to advance science on regulation of dermal sensitization
- Understanding of dermal sensitization of a chemical relies on results of available *in vivo* human and animal studies, as well as available *in vitro* studies
  - Adverse outcome pathway for dermal sensitization has been well defined (*The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins*; OECD 2014, https://www.oecd.org/env/the-adverse-outcome-pathway-for-skin-sensitisation-initiated-by-covalent-binding-to-proteins-9789264221444-en.htm)

EPA and quantification of dermal sensitization

- EPA has presented use of quantitative endpoints for dermal sensitization in previous FIFRA scientific advisory panel (SAP) on hexavalent chromium in 2004 and to HSRB for establishing a point of departure for methylisothiazolinone (MIT) in 2017
- The 2004 SAP supported the agency's interest in developing methods for quantitation of dermal sensitization risk and supported the effort
  - Given that sensitization responses are based on dose/surface area, the Panel concluded that both the Minimum Elicitation Threshold (MET) and Local Lymph Node Assays (LLNA) exposure methodologies are appropriate for collecting sensitization data, as these approaches use dose/unit area.
  - The Panel strongly agreed that, given that the threshold for induction (non-sensitized individuals) is considered to be higher than that required for elicitation (sensitized individuals), establishing a safe level below the threshold for elicitation would also be protective of induction. This is in agreement with existing scientific literature (Kimber et al., 2003).
- In 2017, the HSRB supported the quantitative use of the presented studies in the establishment of a point of departure for MIT

Current stance from other regulatory agencies

Regulatory Body/Country	Stance on Formaldehyde Dermal Sensitization
European Chemicals Agency (ECHA) (current website)	3 µg/cm² in subjects allergic to formaldehyde and 37 µg/cm² for induction of formaldehyde contact dermatitis based on human data **
European Commission Scientific Committee on Consumer Safety (May 2021)	Suggest reducing the present threshold by a factor of 50, that is, to 0.001% (10 ppm), to protect consumers sensitized to formaldehyde.
Proposal for Harmonized Classification and Labelling (CLH) report, Germany, June 2021	Provide range of concentrations based on guinea pig maximation test, Local Lymph Node Assay (mouse) and human studies**
Canada	Formaldehyde permitted in oral cosmetics at a concentration of 0.1% or less and at a concentration of 0.2% or less in non-oral cosmetics as a preservative only.
Australia	Cite “majority of the products contain formaldehyde at low concentrations (< 0.2%) …may induce skin sensitization at even very low concentrations… dermal exposure should be minimized or prevented wherever possible.”

** Utilizes Flyvholm and Fischer

Dermal sensitization/Allergic Contact Dermatitis (ACD)

Characterized by two phases:

- Induction/Sensitization:
  - Exposure of sufficient magnitude and/or duration to activate specific immune mechanisms resulting in the dermal sensitization
- Elicitation/Challenge:
  - Responses induced in sensitized individuals upon subsequent exposure to the allergen

Contact Hypersensitivity*

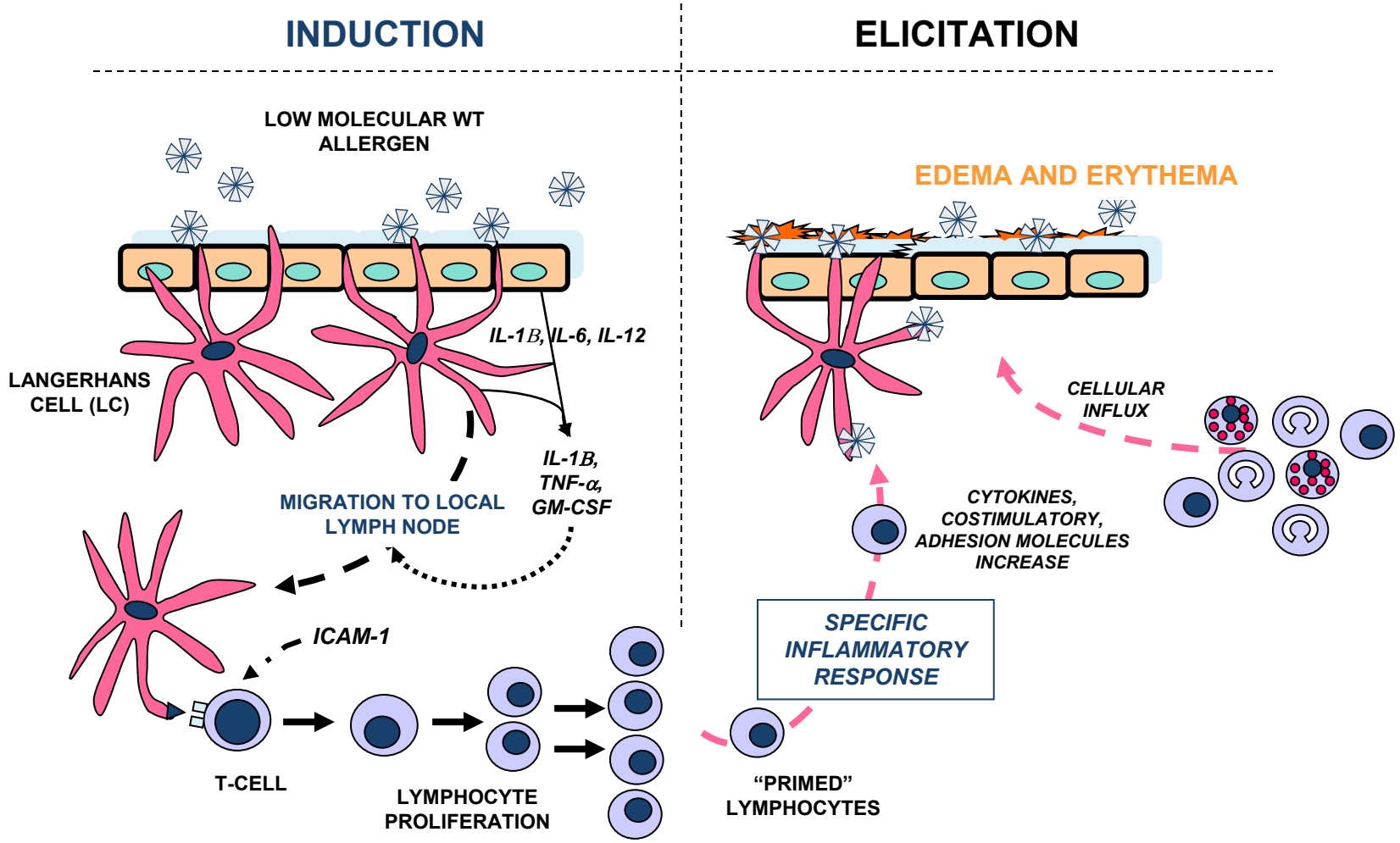


*Illustration by D. Sailstad

Published material

Other data considered by EPA for dermal sensitization by formaldehyde

- Animal data
  - Local Lymph Node Assay (LLNA) and Guinea Pig Maximization Test (GPMT)
  - Tests would be reflective of induction thresholds; less sensitive than elicitation thresholds
- *In vitro* data
  - Sensitization adverse outcome pathway has been well defined
    - Methods developed and utilized in Europe
  - Limited data available on these tests for formaldehyde
    - Search of OPPT identified literature included terms such as hCLAT, IIVS, DPRA, KeratinoSens™ LuSens, ARE-Nrf2 luciferase test, Interleukin-8 Reporter Gene Assay (IL8-Luc)
    - No relevant studies identified from search

Background-Human Studies

- The agency is proposing to use the following studies as part of endpoint selection and point of departure (POD) derivation of an elicitation threshold for dermal sensitivity to formaldehyde. Both studies utilized healthy formaldehyde-sensitive subjects
  - Flyvholm et al., 1997 (*Contact Dermatitis* 36: 26-33)
  - Fischer et al. 1995 (*Current Problems in Dermatology*)
- These studies were identified based on the systematic review conducted by OPPT to identify relevant studies for the assessment of formaldehyde toxicity

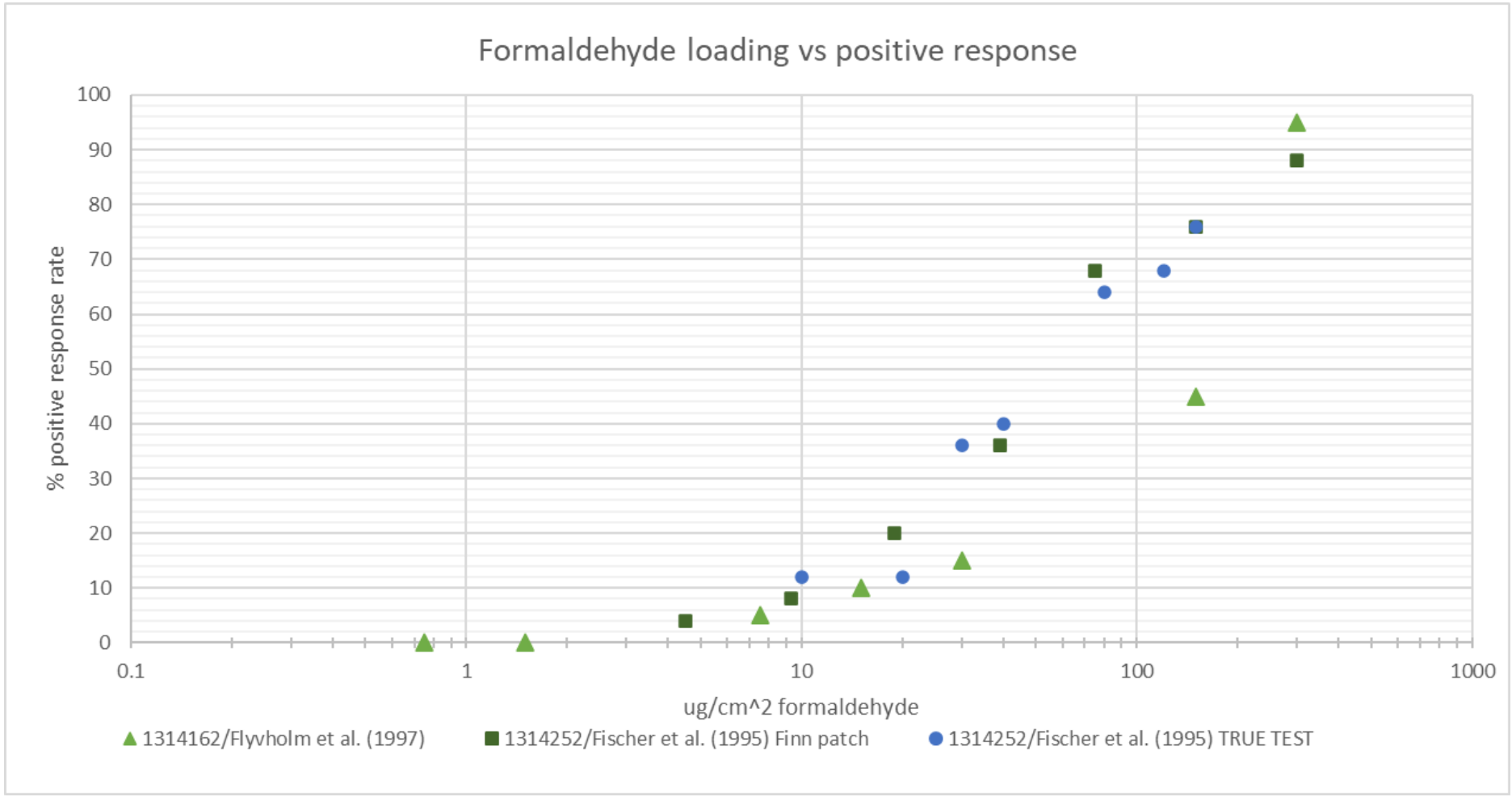
Literature search method for studies on dermal sensitization

- Systematic review process included databases containing publicly available, peer-reviewed literature; gray literature, which is defined as the broad category of data/information sources not found in standard, peer-reviewed literature databases and data; and information submitted under regulatory requirements of either OPPT or OPP
- Most testing for formaldehyde allergies using patch tests generally relies on testing at a concentration of 1% or 2%
  - Conducted generally on non-sensitized individuals
  - Numerous studies captured this testing
- Subset of studies identified where testing was completed in formaldehyde sensitive individuals at lower concentrations in order to determine a “minimum elicitation threshold”
  - These would represent the most sensitive and health protective levels for sensitization
  - EPA focused on this subset of studies

Other available human studies

- Five additional intentional human exposure studies were initially identified that tested concentrations below 1%
- Held et al. (1998), Hauksson et al. (2016), de Groot et al. (1998), Hauksson et al. (2011), Jordan, WP et al. (1979)
- Not included in data set to support quantitative determination of point of departure for various reasons
  - Very limited or no data on quantitative analytical methods
  - Information not provided to estimate skin loading (in units used in dermal risk assessment)
  - Limited study participant information
  - Single dose testing
  - Not relevant to lower range of sensitization/elicitation

Flyvholm et al. and Fischer et al. combined results

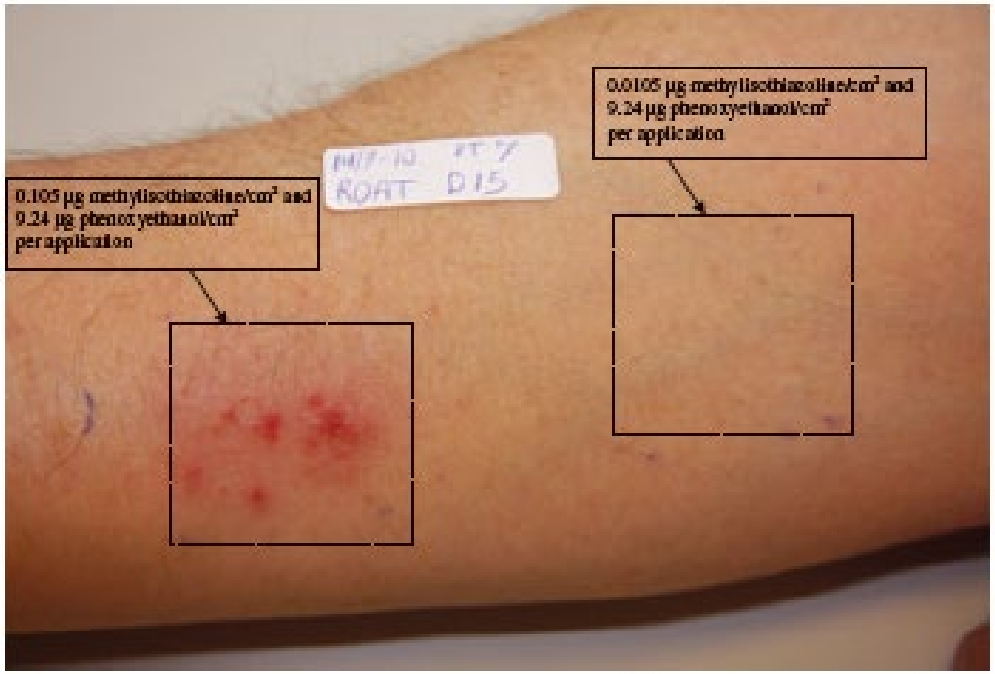


Dermatologic skin testing

- Patch tests
  - Standard used for clinical allergy testing - used to determine sensitivity of an individual to a chemical
  - Typically done by applying the material to a small defined area of the skin (e.g., 0.5 cm^2)
  - Short-term exposures (24-48 hours; may be single or multiple exposures)
  - May be **occluded** (i.e., applied to skin that is covered) or **non-occluded** (i.e., applied to skin that is not covered)

- Repeat Open Application Test (ROAT)
  - Test article is repeatedly applied to a defined area of the skin in sensitized individuals
  - Repeated exposures over longer period of time (usually weeks)
  - Typically a non-occluded test
  - May be considered a more realistic exposure pattern

Patch Test and ROAT Representation

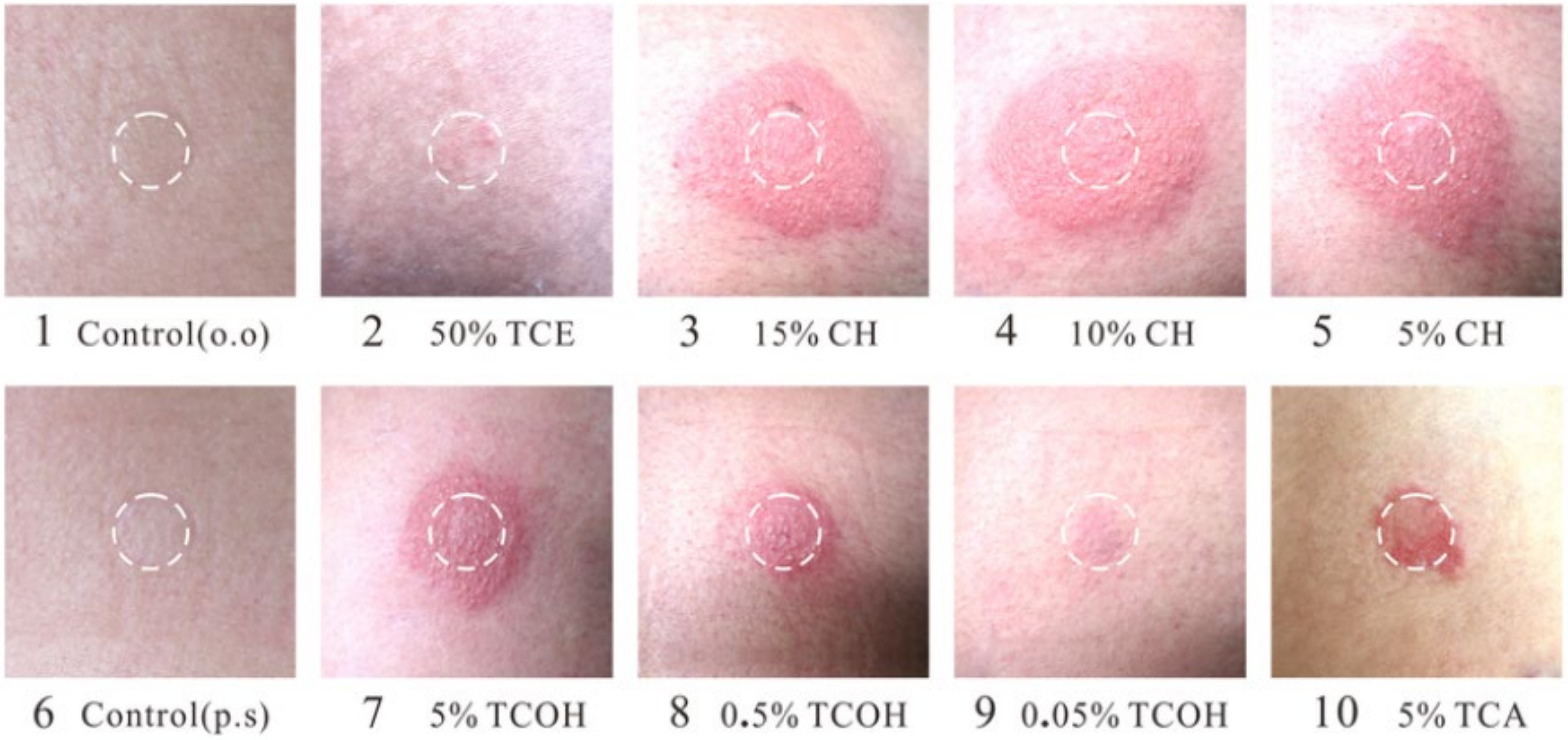


ROAT graphic from Michael D. Lundov, Claus Zachariae and Jeanne D. Johansen (2011): Methylisothiazolinone contact allergy and dose–response Relationships. *Contact Dermatitis* 64: 330-336

Criteria for positive reaction (International Contact Dermatitis Research Group, ICDRG)

Patch test reading	Description
+?	doubtful reaction; faint erythema only
+	weak positive reaction; erythema, infiltration, possibly papules
++	strong positive reaction; erythema, infiltration, papules, vesicles
+++	extreme positive reaction; intense erythema and infiltration and coalescing vesicles
-	Negative reaction
IR	Irritant reaction of different types

Example range of positive reactions across different chemical concentrations



Results of the skin patch test. The white circle represents the circumference of the patch. A positive reaction (++) according to the ICDRG scoring system was observed for chloral hydrate (15, 10 and 5% in olive oil) and trichloroethanol (5% in olive oil). A weak positive reaction (+) was observed for TCE (50%), trichloroethanol (0.5 and 0.05% in olive oil) and trichloroacetic acid (5% in physiological saline). The reactions for TCE at each concentration (25, 10 and 5% in olive oil) and controls were negative.

Graphic from: Huang , Y. et al. 2015. Trichloroethylene Hypersensitivity Syndrome Is Potentially Mediated through Its Metabolite Chloral Hydrate. PLoS ONE 10(5):e0127101

Threshold for occluded formaldehyde patch test in formaldehyde-sensitive patients

Flyvhom, MA, Hall, BM, Agner, T, Edemann, ET, Greenhill, P, Vanderveken, W, Freeberg, FE And T Menne. 1997. Contact Dermatitis. 36: 26-33.

Flyvholm et al. 1997

- OPP made multiple attempts to request the raw data and documentation of the ethical conduct of the study and did not receive responses
- Purpose of this study
  - Investigate the eliciting threshold concentration of formaldehyde in formaldehyde-sensitive individuals in occluded and non-occluded patch tests
  - Evaluate the relationship to repeated open application test (ROAT) with a product containing a formaldehyde releaser
- Used a series of test concentrations for the occluded and non-occluded patch tests
- ROAT conducted at one concentration with formaldehyde releaser

Flyvholm et al. 1997

- 36 formaldehyde-sensitive patients were recruited for study
  - 20 patients agreed to participate in the study (16 refused)
    - 14 women and 6 men; age range 32 - 71 years
    - All had previous positive patch tests to formaldehyde
- Control group consisted of 20 healthy volunteers with negative patch tests to formaldehyde and other test materials
    - 12 women and 8 men; age range 22 - 54 years

Flyvholm et al. 1997

- Occluded and non-occluded patch tests
  - Conducted with formaldehyde solutions in concentrations of 0, 25, 50, 250, 500, 1,000, 5,000 and 10,000 ppm (equivalent to 0, 0.0025 %, 0.0050 %, 0.025 %, 0.050 %, 0.1 %, 0.5 %, and 1 %)
  - ROAT for 1 week with a leave-on cosmetic product containing on average 300 ppm (equivalent to 0.03 %) formaldehyde
- Concentration of the formaldehyde in solutions for occluded and non-occluded patch tests was analyzed by an iodine titration method
- Free formaldehyde and total formaldehyde for ROAT was analyzed by an HPLC method

Flyvholm et al. 1997

- Occluded patch testing
  - 15 µl by Finn Chambers (diameter 0.8 cm)
  - Applied to upper back
  - Tests applied for 2 days; readings performed after 2, 3 and 6-9 days
- Non-occluded patch testing
  - 15 µl of formaldehyde solutions applied to a 1 cm^2 area
  - Applied to area of the forearm; allowed to dry at room temperature
  - Reading times same as occluded
- ROAT
  - Patients applied test material to a 5 x 5 cm area of the flexor mid-aspect of the left upper arm
  - Applied 2x daily for a maximum period of 1 week
  - Readings performed after 1 week

Flyvholm et al. 1997

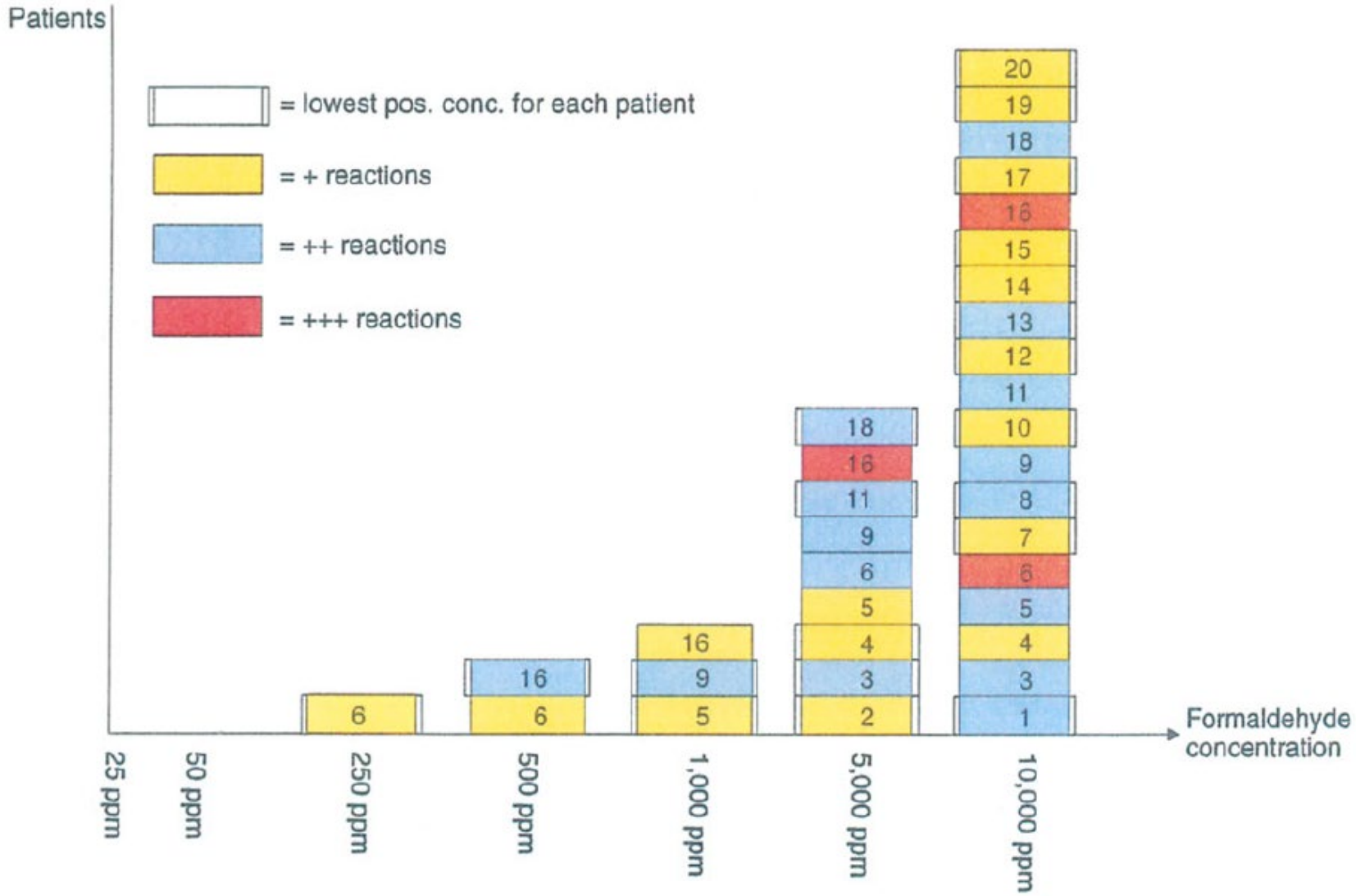
- Criteria for positive reaction (International Contact Dermatitis Research Group, ICDRG)

Patch test reading	Description
+?	doubtful reaction; faint erythema only
+	weak positive reaction; erythema, infiltration, possibly papules
++	strong positive reaction; erythema, infiltration, papules, vesicles
+++	extreme positive reaction; intense erythema and infiltration and coalescing vesicles
-	Negative reaction
IR	Irritant reaction of different types

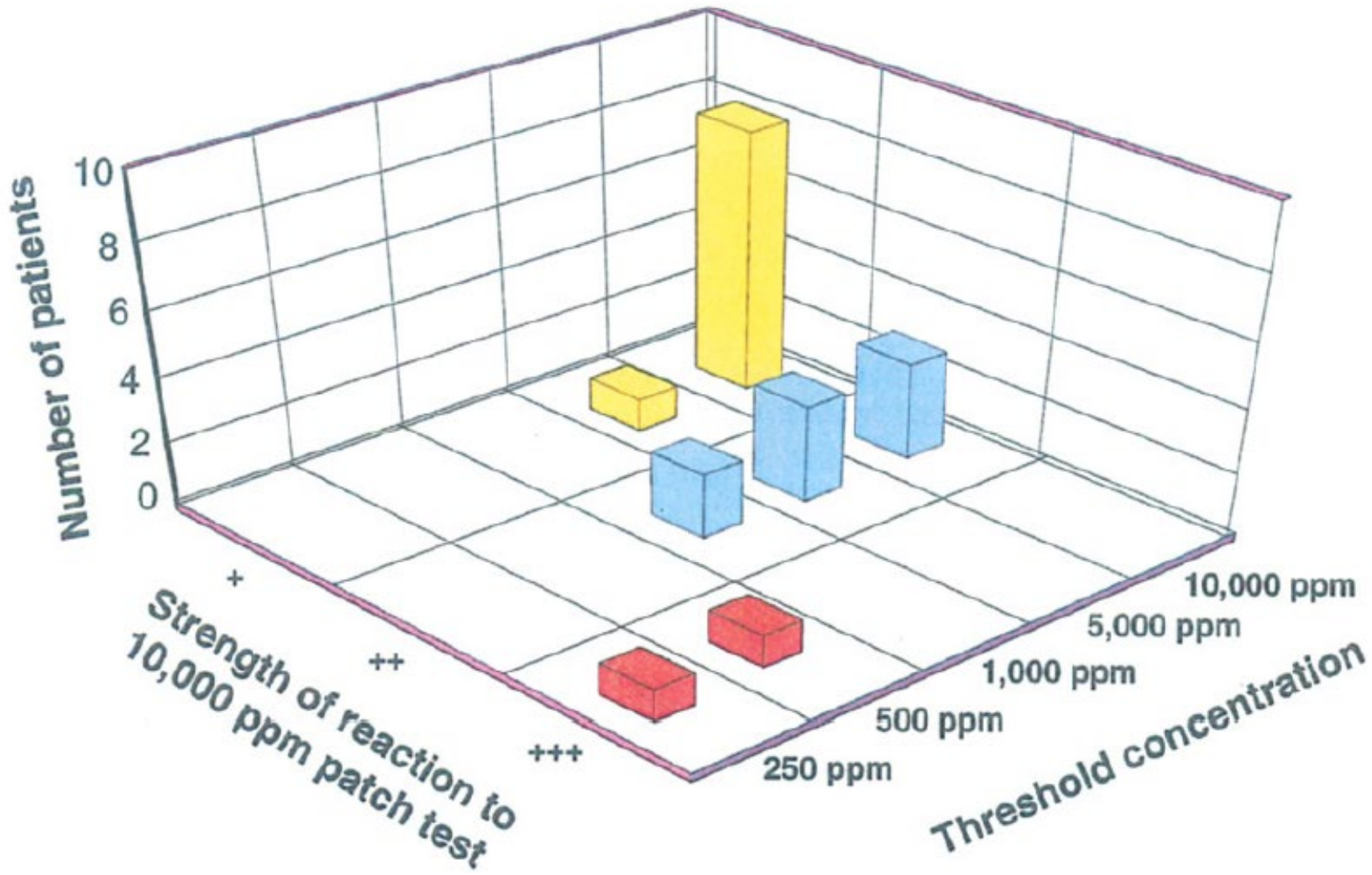
Flyvholm et al. 1997: Occluded patch test results

Concentration tested (in varying units)[1]			Positive results (% of tested)
ppm	%	µg/cm2	
10,000	1	300	19/20 (95 %)
5,000	0.5	150	9/20 (45 %)
1,000	0.1	30	3/20 (15 %)
500	0.05	15	2/20 (10 %)
250	0.025	7.5	1/20 (5 %)
50	0.005	1.5	0/20
25	0.0025	0.75	0/20
0 (Control)	0	0	0/20

Flyvholm et al. 1997: Occluded patch test results (Figure 1 from study)



Flyvholm et al. 1997: Occluded patch test results (Figure 2 from study)



Flyvholm et al. 1997

- Non-occluded patch testing results
  - No positive reactions based on the established criteria
  - In 6 out of 20 patients, weak reactions showing erythema without infiltration or follicular reactions were observed (did not meet criteria)
- ROAT
  - No positive reactions based on the established criteria
  - Few follicular papules were observed in 5 of 20 patients
  - Concentration of free formaldehyde in the ROAT cream was 300 ppm, but the actual dose varied across subjects from 0.71 µg/cm^2 to 2.91 µg/cm^2 (low end of patch test dose range)
    - Variability in dose due to subjects applying varying amounts of cream

Flyvholm et al. 1997

- EPA's attempts to obtain the raw data from the study authors were unsuccessful
- EPA in conjunction with our statistics contractor ICF, reviewed and attempted to reproduce any statistical analyses described in the studies
- No additional statistical analyses were feasible for the study based on the lack of reported raw data

Strengths/limitations of Flyvholm et al. 1997

Strengths:

- Adequate number of participants in this study
- Both males and females are represented in the study
- Individuals with previously confirmed sensitivity to formaldehyde participated
- Information on degree of response provided
- Experimental design to examine dose-response relationship for elicitation threshold for formaldehyde; a NOAEL/LOAEL can be identified.
- Skin loading in Flyvholm et al aligns with potential skin loading from expected uses (e.g., FIFRA registered uses at 370 ppm formaldehyde, loading estimates approximately 3.8 µg/cm^2)

Strengths/limitations of Flyvholm et al. 1997

Limitations:

- Limited information was provided on the test substance, including the purity or source of formaldehyde or if stabilizers were present (such as methanol)
  - Methanol is an irritant but not a known dermal sensitizer
  - Formaldehyde commonly formulated with stabilizers present, so may represent actual exposures
- Reading day not reported for individual results (2, 3 or 6-9 days)
- Concentrations reported as measured/confirmed, but unclear if nominal or measured concentrations are used in the study

Overall Conclusions – Flyvholm et al. 1997

- Based on the concentrations tested in the occluded patch tests, the Minimum Elicitation Threshold or Lowest Observed Adverse Effect Level (LOAEL) was 250 ppm (0.025 % or 7.5 µg/cm^2), and the No Observed Adverse Effect Level (NOAEL) was 50 ppm (0.005 % or 1.5 µg/cm^2)

- The study was well-conducted and provides quantitative information for deriving a minimum elicitation threshold for formaldehyde such that it can be considered as part of endpoint selection and POD derivation

Charge Question

Is the research described in the published study *"Flyvholm, MA, Hall, BM, Agner, T, Tiedemann, E, Greenhill, P, Vanderveken, W, Freeberg, FE and T Menné. (1997). Threshold for Occluded Formaldehyde Patch Test in Formaldehyde-Sensitive Patients. Contact Dermatitis. 36: 26-33"* scientifically sound, providing reliable data such that it can be considered as part of endpoint selection and POD derivation for elicitation of dermal sensitization from dermal exposure?