Registration Decision RD2014-02

2-Methyl-4-isothiazolin-3-one

(publié aussi en français)

6 March 2014

This document is published by the Health Canada Pest Management Regulatory Agency. For further information, please contact:

Publications
Pest Management Regulatory Agency
Health Canada
2720 Riverside Drive
A.L. 6604-E2
Ottawa, Ontario K1A 0K9

Internet: pmra.publications@hc-sc.gc.ca
healthcanada.gc.ca/pmra
Facsimile: 613-736-3758
Information Service:
1-800-267-6315 or 613-736-3799
pmra.infoserv@hc-sc.gc.ca

Canada

ISSN: 1925-0932 (print)
1925-0940 (online)

Catalogue number: H113-25/2014-02E (print version)
H113-25/2014-02E-PDF (PDF version)

Table of Contents

Registration Decision for 2-Methyl-4-isothiazolin-3-one

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is granting full registration for the sale and use of Kordek 573T Technical Microbicide, Kordek 573F Industrial Microbicide, Kordek LX 5000 Industrial Microbicide, Kordek MLX Industrial Microbicide, Rocima 550 Industrial Microbicide and Neolone M-10 Industrial Microbicide, containing the technical grade active ingredient 2-methyl-4-isothiazolin-3-one, to be used as a material preservative in paint, coatings, metal-working fluids, household products and polymer latices.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

These products were first proposed for registration in the consultation document[1] Proposed Registration Decision PRD2011-02, *2-Methyl-4-isothiazolin-3-one*. This Registration Decision[2] describes this stage of the PMRA's regulatory process for 2-methyl-4-isothiazolin-3-one and summarizes the Agency's decision, the reasons for it, and provides, in Appendix I, a summary of comments received during the consultation process as well as the PMRA's response to these comments. This decision is consistent with the proposed registration decision stated in PRD2011-02.

For more details on the information presented in this Registration Decision, please refer to PRD2011-02, which contains a detailed evaluation of the information submitted in support of this registration.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[3] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its conditions of registration. The Act also requires that products have value[4] when used according to label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

---

1 "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act.*

2 "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act.*

3 "Acceptable risks" as defined by subsection 2(2) of *Pest Control Products Act.*

4 "Value" as defined by subsection 2(1) of *Pest Control Products Act*"...the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (*a*) efficacy; (*b*) effect on host organisms in connection with which it is intended to be used; and (*c*) health, safety and environmental benefits and social and economic impact".

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (for example, children) as well as organisms in the environment (for example, those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides and Pest Management portion of Health Canada's website at healthcanada.gc.ca/pmra.

What Is 2-Methyl-4-isothiazolin-3-one?

The compound 2-methyl-4-isothiazolin-3-one is a new active ingredient proposed for use as an in-container preservative to prevent bacterial spoilage in polymer latices, metal-working fluids, mineral slurries, paints, detergents, cleaners, and polishes. This active ingredient is a broad-spectrum biocide that acts by disrupting microbial metabolism. While the combination of 2-methyl-4-isothiazolin-3-one and 5-chloro-2-methyl-4-isothiazolin-3-one has been registered as an integrated system product for in-can preservation of a number of materials, the use of 2-methyl-4-isothiazolin-3-one alone constitutes a new active ingredient.

Health Considerations

Can Approved Uses of 2-methyl-4-isothiazolin-3-one Affect Human Health?

2-Methyl-4-isothiazolin-3-one is unlikely to affect your health when used according to label directions.

Potential exposure to 2-methyl-4-isothiazolin-3-one may occur when handling and applying the product or through contact with materials containing the product as a preservative. When assessing health risks, two key factors are considered: the levels where no health effects occur and the levels to which people may be exposed. The dose levels used to assess risks are established to protect the most sensitive human population (for example, children and nursing mothers). Only uses for which the exposure is well below levels that cause no effects in animal testing are considered acceptable for registration.

Toxicology studies in laboratory animals describe potential health effects from varying levels of exposure to a chemical and identify the dose where no effects are observed. The health effects noted in animals occur at doses more than 100-times higher (and often much higher) than levels to which humans are normally exposed when 2-methyl-4-isothiazolin-3-one products are used according to label directions.

In laboratory animals, 2-methyl-4-isothiazolin-3-one was of high acute toxicity by the oral and dermal route, and of moderate acute toxicity via the inhalation route. 2-Methyl-4-isothiazolin-3-one was corrosive to the eyes and to the skin, and caused an allergic skin reaction. Consequently, the hazard signal words “DANGER – POISON, CORROSIVE TO EYES AND SKIN, POTENTIAL SKIN SENSITIZER” are required on the label. End-use products containing 2-methyl-4-isothiazolin-3-one have similar acute toxicity and require the same hazard signal words on their label.

2-Methyl-4-isothiazolin-3-one did not cause cancer in animals and is unlikely to damage genetic material. There was no indication that 2-methyl-4-isothiazolin-3-one caused damage to the nervous system and concerns for adverse effects on the immune system were low. 2-Methyl-4-isothiazolin-3-one did not cause birth defects in animals. Health effects in animals given repeated doses of 2-methyl-4-isothiazolin-3-one included effects on body weight, body weight gain and food consumption and irritation at the site of contact (skin, stomach or nasal cavity/lungs) as well as slight changes in blood parameters.

When 2-methyl-4-isothiazolin-3-one was given to pregnant rabbits, effects of a serious nature (increased incidence of embryo/foetal loss in the developmental toxicity study) were observed at doses that were toxic to the mother. Changes in organ weights as well as delayed sexual maturation and slight decreases in the number of live births were also observed at doses that were toxic to the mother in the rat reproduction study. The risk assessment takes these effects into account in determining the allowable level of human exposure to 2-methyl-4-isothiazolin-3-one.

The risk assessment protects against the effects of 2-methyl-4-isothiazolin-3-one by ensuring that the level of human exposure is well below the lowest dose at which these effects occurred in animal tests.

Residues in Water and Food

No food uses were proposed with this application, therefore a food residue assessment was not required.

Risks in Residential and Other Non-Occupational Environments

Estimated risk for non-occupational exposure is not of concern.

A quantitative risk assessment conducted for individuals using paints and cleaning products, containing Kordek LX 5000 Industrial Microbicide, Kordek MLX Industrial Microbicide, Rocima 550 Industrial Microbicide or Neolone M-10 Industrial Microbicide, indicated that the risk is not of concern.

Risks in Secondary Occupational Environments

Estimated occupational risks to secondary workers are not of concern.

Secondary workers can come in direct contact with 2-methyl-4-isothiazolin-3-one on the skin or through inhalation while working with paints, cleaning products or metal-working fluids.

Quantitative risk assessments were conducted for individuals handling Kordek LX 5000 Industrial Microbicide, Kordek MLX Industrial Microbicide, Rocima 550 Industrial Microbicide or Neolone M-10 Industrial Microbicide, which indicated that the risk for workers is not of concern when handling paints, cleaning products or metal-working fluids.

Occupational Risks From Handling Kordek LX 5000 Industrial Microbicide, Kordek MLX Industrial Microbicide, Rocima 550 Industrial Microbicide or Neolone M-10 Industrial Microbicide

Occupational risks are not of concern when Kordek LX 5000 Industrial Microbicide, Kordek MLX Industrial Microbicide, Rocima 550 Industrial Microbicide or Neolone M-10 Industrial Microbicide are used according to the proposed label directions, which include protective measures.

A quantitative risk assessment conducted for individuals handling Kordek LX 5000 Industrial Microbicide, Kordek MLX Industrial Microbicide, Rocima 550 Industrial Microbicide or Neolone M-10 Industrial Microbicide indicated that the risk for workers is not of concern when these products are used according to label directions.

Workers mixing and loading Kordek LX 5000 Industrial Microbicide, Kordek MLX Industrial Microbicide, Rocima 550 Industrial Microbicide or Neolone M-10 Industrial Microbicide can come in direct contact with 2-methyl-4-isothiazolin-3-one on the skin or through inhalation. Therefore, the label will specify that workers must wear coveralls over a long-sleeved shirt and long pants, chemical-resistant gloves, socks, chemical-resistant footwear and a full face NIOSH-approved respirator when mixing and loading Kordek LX 5000 Industrial Microbicide, Kordek MLX Industrial Microbicide, Rocima 550 Industrial Microbicide or Neolone M-10 Industrial Microbicide.

Environmental Considerations

What Happens When 2-Methyl-4-isothiazolin-3-one Is Introduced Into the Environment?

2-Methyl-4-isothiazolin-3-one is the active ingredient in a number of end-use products, which will be used as a material preservative in paint, coatings, metal-working fluid, household products and polymer latices. Based on the proposed use pattern, terrestrial and aquatic environmental exposure is expected to be minimal. 2-Methyl-4-isothiazolin-3-one and its four major transformation products are categorized as non persistent to slightly persistent in aerobic soil. In laboratory studies 2-methyl-4-isothiazolin-3-one is stable to hydrolysis; however, based on its chemical structure and low concentration during use, it is expected to be susceptible to microbial degradation in the aquatic environment (including water/sediment systems), resulting in negligible concentrations in water bodies. Based on rapid dissipation in soil, concentrations in groundwater are expected to be low.

Under the use pattern proposed, 2-methyl-4-isothiazolin-3-one is not expected to present a risk to wild mammals, birds, freshwater or marine invertebrates and fish, amphibians, algae, and aquatic and terrestrial plants.

Value Considerations

What is the value of 2-methyl-4-isothiazolin-3-one and the end-use products Kordek LX 5000 Industrial Microbicide, Kordek MLX Industrial Microbicide, Rocima 550 Microbicide Industrial, and Neolone M-10 Industrial Microbicide?

As a broad-spectrum biocide, 2-methyl-4-isothiazolin-3-one acts to inhibit the growth of spoilage microorganisms within a number of aqueous-based materials.

When used according to label instructions, Kordek LX 5000 Industrial Microbicide, Kordek MLX Industrial Microbicide, Rocima 550 Industrial Microbicide, and Neolone M-10 Industrial Microbicide are able to provide effective in-can protection to a number of aqueous-based materials. These end-use products, when added to polymer latices, metal-working fluids, mineral slurries, paints, detergents, cleaners, and polishes at rates ranging from 25-150 ppm active ingredient, were able to provide effective protection against a broad range of bacteria, mould and yeast. Without a preservative, these materials supported abundant microbial growth, which may lead to foul odours, discoloration, pH changes and destabilization of the product formulation. For a number of materials, such as metal-working fluids and paints, where there is the potential to introduce spoilage bacteria multiple times over the life of the product from opening and closing the container, data was provided to show that the end-use products continued to provide protection against multiple inoculations. While there are a number of different active ingredients currently registered as in-container preservatives for susceptible materials, 2-methyl-4-isothiazolin-3-one provides an alternative option that may be useful to address future cost, availability or microbial resistance issues.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The key risk-reduction measures being proposed on the labels of Kordek LX 5000 Industrial Microbicide, Kordek MLX Industrial Microbicide, Rocima 550 Industrial Microbicide, and Neolone M-10 Industrial Microbicide to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health
Anyone handling Kordek LX 5000 Industrial Microbicide, Kordek MLX Industrial Microbicide, Rocima 550 Industrial Microbicide, and Neolone M-10 Industrial Microbicide, in an occupational setting, must wear all the personal protective equipment as stated on the label.

Environment
Label statements for toxicity will be required for aquatic organisms.

Other Information

The relevant test data on which the decision is based (as referenced in PRD2011-02) are available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa). For more information, please contact the PMRA's Pest Management Information Service by phone (1-800-267-6315) or by e-mail (pmra.infoserv@hc-sc.gc.ca).

Any person may file a notice of objection[5] regarding this registration decision within 60 days from the date of publication of this Registration Decision. For more information regarding the basis for objecting (which must be based on scientific grounds), please refer to the Pesticides and Pest Management portion of the Health Canada's website (Request a Reconsideration of Decision, healthcanada.gc.ca/pmra) or contact the PMRA's Pest Management Information Service.

[5] As per subsection 35(1) of the *Pest Control Products Act.*

Appendix I Comments and Responses

Repeat Dose Toxicity

1. The applicant noted that as several of the endpoints selected for risk assessment were based on studies conducted with CMIT/MIT (Kathon), more consideration should be given to CMIT's increased toxicity and reactivity relative to MIT (Kordek). The applicant also noted that the toxicity of CMIT is primarily based on irritation at the site of contact, which they contend is not representative of systemic toxicity.

Response: For a significant number of DACOs, the applicant chose to bridge to toxicity data from the Kathon database. The greater reactivity of CMIT is a cornerstone of why this bridge was considered to be acceptable. Also, the PMRA does not agree that toxicity was limited to local irritation, and has noted systemic toxicity in both the Kathon and Kordek toxicology studies (as further detailed below).

2) The applicant indicated that there was no evidence of systemic toxicity in the 90-day rat (drinking water) and dog (dietary) studies conducted with MIT. The decreases in body weight gain and food consumption were considered by the applicant to be secondary to decreased water consumption, and other changes such as those in clinical chemistry and hematology parameters were considered to be minimal/non-adverse.

Response: The PMRA noted systemic effects in these 90-day studies in the rat (via drinking water) and dog (via diet). In the rat, these included effects on body weight and body weight gain, decreases in food and water consumption, and decreased plasma glucose and bilirubin levels. Effects on body weight and body weight gains are often one of the most sensitive indicators of toxicity. A strong scientific rationale, backed by evidence, would be required before these findings could be dismissed as being secondary to decreased water consumption.
In dogs (notwithstanding the issues noted below) again decreases in body weight, body weight gain and food consumption were noted. The presence of body weight effects in a dietary (as opposed to water based) study lends further support to body weight effects being a primary effect of MIT toxicity. Furthermore, calcium levels were also decreased in the dog.

The PMRA agrees that some of the haematological findings in these 90-day studies were not of sufficient magnitude to be considered adverse. For example, in the rat study the effects on red blood cell counts, haemoglobin and hematocrit were considered treatment related but not adverse. However, in the dog study reductions of approximately 10% in red blood cell counts, haemoglobin and hematocrit were noted. These reductions are considered to be biologically relevant and adverse, and also considered to be further evidence of systemic toxicity.

3. According to the applicant, findings in the 3- and 24-month rat drinking water studies conducted with Kathon were limited primarily to local irritation, and any evidence of systemic toxicity was suggested to be secondary to decreases in water consumption. The applicant also indicated that it is not appropriate to express irritation endpoints on a mg/kg bw/day basis for subsequent use in risk analysis for systemic toxicity.

Response: The PMRA disagrees with the position that there was no evidence of systemic toxicity in the 3- and 24-month drinking water studies conducted with Kathon. At the mid- and high-doses in the 24-month study, effects on body weight, body weight gain and food consumption were noted, which as indicated above cannot be dismissed as being secondary to decreased water consumption in the absence of a scientifically valid rationale. Systemic toxicity was also noted in the 3-month Kathon study (including body weight effects, clinical chemistry findings, liver and kidney weight changes, in addition to stomach irritation). It was not considered necessary to further comment on the point regarding the appropriateness of using an irritation endpoint for assessing systemic toxicity risks since the findings in these studies were considered by the PMRA to be indicative of systemic toxicity.

4. The applicant suggested that since CMIT is relatively more toxic than MIT, a reduction in the overall uncertainty factor should be considered when using CMIT data in the MIT risk assessment.

Response: As noted previously, the fact that there was evidence that Kathon (containing CMIT) was of greater relative toxicity compared to MIT formed the basis for accepting the bridging rationale for the use of Kathon toxicity data to supplement the MIT database. The greater toxicity of CMIT cannot also be used as justification to lower uncertainty factors in the risk assessment. This is because bridging to another product generally introduces a level of uncertainty, given that the full complement of required studies on the chemical of interest is not available. Further, it is not uncommon for there to be some differences noted in the toxicity of the two compounds in the studies that are available. For example, differences in reproductive toxicity findings were noted between Kathon and Kordek. Moreover, there was some evidence from the acute toxicity data indicating that the greater relative toxicity of CMIT compared to MIT may not be consistent via all routes of exposure.

90-Day Dog Study

5. The applicant disagreed with the PMRA's decision of not considering the data from the 2004 dog study conducted on Kordek, and also noted that analytical recovery of MIT from the diet was only an issue at the lowest concentration tested (with 74% recovery), and notes that the low dose did not play a role in the selection of the no observed adverse effect level (NOAEL) in the study.

Response: The PMRA wishes to clarify that the data from the 2004 dog study were indeed considered during the review, but that the study was found to be unacceptable. Further, the stated 74% recovery value is not the reason that the PMRA considered the study unacceptable. Instead, the PMRA was concerned with the protocols used to determine the recovery values, noting mathematical errors, discrepancy in volume used etc. In addition, the report did not provide sufficient raw data to confirm the dose validation, which is a crucial part of any study. As MIT has the capacity to bind to protein, the quantification of recovery of test material from the diet was important. The PMRA communicated these concerns to the applicant. Although the applicant did eventually provide an amended report to alleviate these concerns, it was submitted well after the date of publication of this PRD, and as such the study was not acceptable at said date. It should be noted that ultimately the study was not found to be pivotal in the overall risk assessment.

Inhalation Toxicity

6. The applicant indicated that the effects noted in the 90-day inhalation study were all consistent with local contact irritation of the respiratory tract, and that the findings noted at the high dose (including effects on the spleen, serum protein and body weight gains) were secondary to this local irritation.

Response: Body weight decreases were consistently seen throughout the database, and as explained above, are not considered to be a secondary effect of MIT or CMIT/MIT.

7. The applicant suggested that using inhalation data for Kathon as a surrogate for MIT is very conservative since Kathon with the CMIT component is much more irritating and biologically reactive than MIT alone. Data from Respiratory Depression 50% (RD50) studies were also referenced to support this contention. Consideration should be given to reducing the overall uncertainty factor in light of Kathon's increased irritation to the respiratory tract compared to MIT.

Response: It was noted that, unlike the oral acute toxicity, the acute inhalation toxicity of MIT is roughly equal to CMIT/MIT, indicating that there may be less differences in the toxicity of these two compounds via the inhalation route than there exists via other routes. Comments relating to a reduction of the overall uncertainty factor have already been made.

8. The applicant stated that the additional proposed uncertainty factor of 3-fold for extrapolation from short-term to long-term inhalation exposure, and conversion to a systemic dose, is not needed, since the only effects observed in the Kathon 90-day inhalation study were from irritation of the respiratory tract. The applicant also indicated that local contact irritation effects are concentration-dependent and not time-dependent, meaning that the irritation effects do not worsen with time if exposure is kept below non-irritating levels.

Response: The 90-day inhalation study was chosen for the long-term inhalation toxicological endpoint as it was conducted via the relevant route of exposure and because its NOAEL was far lower than the 24-month oral Kathon study. Chronic exposure to a respiratory irritant can lead to various health effects that are more debilitating than mere respiratory irritation observed following shorter-term exposures. Furthermore, systemic effects were noted in this respiratory study as noted above. Body weight gain is a consistent finding throughout the database and cannot be dismissed as secondary to local irritation without further evidence. Finally, durational effects were noted for findings in Kathon studies – the PMRA notes for instance the 2.5 fold decrease in NOAELs between the 90-day and the 24-month oral studies. Such evidence of durational effects warrants the application of an additional factor when using a short-term study in lieu of a chronic one according to standard PMRA policy.

The PMRA notes that an inhalation study performed with Kordek would better inform the risk assessment. A study of the appropriate duration would be required to address the issue of durational effects via the inhalation route of exposure.

9. As noted in the applicant's comments, and also in further discussion with the PMRA regarding the 90-day inhalation study, the applicant indicated concerns as to the scientific validity of converting a concentration value that produced a point of contact effect to a systemic value (mg/kg bw/day).

Response: The PMRA acknowledges that there is merit in this point made by the applicant, but does not believe that the approach used by the PMRA is unduly conservative (based on breathing patterns, surface and mass ratios between human and rats etc.).

The PMRA is aware of, and currently examining, other approaches to inhalation risk assessments, such as that taken by the United States Environmental Protection Agency (USEPA) in which a dosimetric adjustment is applied to convert the exposure concentration in animal studies to human equivalent concentrations[a]. No changes to the current PMRA approach have been made at the current time, however.

Reproductive Toxicity

10. The applicant strongly disagrees with the PMRA's conclusion that there were reproductive effects in the 2-generation reproduction study. The applicant's concerns related particularly to the PMRA's interpretation of the following endpoints: a) body weight and food consumption effects, b) organ weight changes, c) decreases in implantation sites, and d) delays in sexual maturation. Some of these endpoints were considered by the applicant to be secondary to decreased water consumption or body weight changes, and others to be within the historical control data ranges and/or not statistically significant, as further detailed below.

Response: The PMRA concluded that the submitted two generation reproductive toxicity study for MIT showed evidence of maternal, reproductive, and offspring toxicity at the highest dose tested (1000 ppm). It should be noted that route-relevant historical control data were not provided.

a) It was indicated by the applicant that decreases in parental body weights and food consumption were secondary to decreased water consumption.

This position is not accepted, as indicated previously for the other toxicology studies.

b) The applicant indicated that changes in organ weights were generally secondary to decreased body weights, and in many cases the organ weights fell within the range of historical controls. Further, it was noted that in many cases the changes noted by the PMRA were to relative weights, and there were no corresponding changes in absolute weights or histopathology.

The changes in absolute ovary and pituitary weights are considered by the PMRA to be treatment-related since 1) it is unlikely that pituitary and ovary weights vary proportionately to changes in body weight, 2) both sexes and/or both generations showed decreased absolute pituitary and ovary weights, albeit slight, and most importantly 3) a functional correlation was observed with the decreased ovarian and pituitary weights: decreased implantations and delayed sexual maturation. Therefore, although not statistically significant, the observed decreases in absolute ovary and pituitary weights are considered biologically relevant.

In F_1 females, absolute and relative uterine weights were increased at the high dose. This effect was supported by an increase in luminal distention in the uterus suggesting that it was treatment-related. Although not statistically significant, these effects are considered biologically relevant. Possible treatment-related effects were noted by the PMRA for the prostate, seminal vesicles and cauda epididymis based on increases in the relative weights of these tissues.

Since most reproductive organ weight changes were observed in multiple generations and/or sexes, and in conjunction with decreased fertility in the F_1 generation and delayed attainment of sexual maturity in those same animals, it lends further support to their being treatment-related at the high dose.

It appears that an error was made in the text of the PRD regarding there being an effect of treatment on kidney weights in parental animals. The PMRA agrees that the increases in relative kidney weights are more likely secondary to body weight decreases.

The PMRA does not agree that there was sufficient evidence to conclude that changes in thymus and spleen weights in offspring were secondary to changes in body weight. In the F_2 pups, both absolute and relative spleen weights were decreased in females at the high dose. Thymus weights, both absolute and relative, were decreased at the high dose in F_1 females. In the F_2 pups, thymus weights were decreased at the high dose in both sexes. In all cases, absolute changes were statistically significant. Although these tissues were preserved for a possible future examination, histopathology was not conducted on spleen or thymus as part of this project. Therefore, these effects are considered treatment-related. Further, although the applicant stated that the thymus and spleen weight values at the high dose fell within the ranges of historical control data, as noted above, this point cannot be validated since route-relevant historical control data were not provided.

c) The applicant did not agree that there were any treatment-related changes in the number of implantation sites or number of pups born in the F_2 generation since there were no statistically significant changes in these parameters compared to controls, the values were within the ranges of historical data, and there were no changes in any other reproductive performance parameters tested.

The mean number of implantation sites for the F_2 generation was decreased at the high dose, with a corresponding decrease in the mean number of F_2 pups born at that dose. Despite their lack of statistical significance, these effects are considered treatment-related and adverse. The applicant indicated that these parameters were within the range of historical data; however, the PMRA notes that since route-relevant historical data were not provided, this point cannot be validated. Further, an enumeration of growing follicles and corpora lutea was not conducted and would have provided a more complete assessment of the effect of MIT on fertility.

d) The applicant suggested that the changes in balanopreputial separation and time to vaginal patency in high dose F_1 pups were associated with decreases in pup weight gain over this period. In addition, they noted that no changes were observed in anogenital distances of either sex in the F_2 pups, which they suggested was a more sensitive parameter.

At the high dose, sexual maturation was delayed in both males and females. Balano-preputial separation and time to vaginal patency were delayed by 2.5 and 2.6 days, respectively. The inference that these changes were due to decreases in pup body weight is not plausible given that the differences in body weights from controls in both males and females were marginal (5.6% decrease in body weight in males and 4.8% decrease in body weight in females). Much more significant changes in body weight are required to elicit a secondary delay in sexual maturation. Thus, the observed delays in sexual maturation are considered adverse and are likely related to the observed decreases in pituitary weights.

The PMRA does not agree that anogenital distance is "a more sensitive parameter" than the sexual maturation parameters discussed above. These endpoints should be considered independently of one another (although they both have endocrine-mediated development).

Overall, regarding the reproductive toxicity study, the PMRA concludes the following:

The parental lowest observed adverse effect level (LOAEL) is 69 mg/kg bw/day in males, 93 mg/kg bw/day in females, based on body weight and organ weight effects. The parental NOAEL is 15 mg/kg bw/day in males, 22 mg/kg bw/day in females.

The reproductive LOAEL is 93 mg/kg bw/day, based on a decreased number of implantation sites and decreased mean number of F_2 pups born. The reproductive NOAEL is 22 mg/kg bw/day.

The offspring LOAEL is 69 mg/kg bw/day in males and 93 mg/kg bw/day in females, based on body weight and organ weight effects, and based on the delayed sexual maturation in both sexes. The offspring NOAEL is 15 mg/kg bw/day in males and 22 mg/kg bw/day in females.

Developmental Toxicity

11. The applicant disagreed with the PMRA's conclusion that MIT was slightly more toxic than CMIT/MIT to maternal animals when comparing the rat developmental toxicity studies, and suggested that the difference in opinion may be related to a misunderstanding of how the doses were expressed in the CMIT/MIT study.

Response: The PMRA thanks the applicant for this clarification. The comment regarding the slightly higher toxicity of MIT to rat dams should have simply said:

> "When comparing the two chemicals in rats, there does not appear to be a significant difference in terms of developmental toxicity."

12. The applicant disagreed with the PMRA's conclusions regarding developmental toxicity effects in the rabbit studies conducted with CMIT/MIT. The applicant noted that "In the first study (conducted prior to guidelines or good laboratory practices), the review by PMRA indicated that CMIT/MIT produced heart malformations; however, in this report these were only observed in the vehicle control/Mg-salt group. Also, PMRA indicated there was an increased incidence of displayed extra ribs and partially ossified sternebra; however, there is a higher

incidence of these findings in the Control and Mg-salt Control groups than any treated group. In the second rabbit developmental study with CMIT/MIT, conducted according to guidelines and utilizing good laboratory practices, the maternal and embryo-fetal NOELS were 2 and 8 mg/kg/day, respectively".

Response: The increased incidences of heart malformation, extra ribs and partially ossified sternebra were observed in the most recent study conducted in 1992, not the 1977 study conducted prior to good laboratory practice guidelines. The maternal and embryo-fetal NOAELS of 2 and 8 mg/kg bw/day were noted in the 1992 study, not the 1977 study. It appears that the applicant and the PMRA used the term "first study" and "second study" to indicate different studies. For greater clarity, the PRD text should have read:

> "In the first study, conducted in 1992, all animals at the high-dose level were..."

And:

> "In the second study, performed in 1977 on a different strain of rabbits, mortality, body weight effects..."

[a]: see "USEPA (1994). Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. United States Environmental Protection Agency, Office of Research and Development, Washington, DC. EPA/600/8-90/066F." and "USEPA (2002). A Review of the Reference Dose and Reference Concentration Processes. United States Environmental Protection Agency, Risk Assessment Forum, Washington, DC. EPA/630/P-02/002F."

Dermal Absorption

13. The applicant expressed concern that no consideration was given to the human in vitro dermal absorption study.

Response: Several NAFTA agencies (PMRA, California Department of Pesticide Regulation [CalDPR] and USEPA) are in agreement that human in vitro dermal absorption studies, alone, are not sufficiently validated for use in deriving estimates of systemic exposure for risk assessment. However, the PMRA and the CalDPR currently use human in vitro data in a limited capacity (for example, bridging data such as the ratio of human to rodent dermal absorption, investigating formulation effects).

The PMRA did review the study submitted by the applicant (In vitro Absorption from Water and Three Formulations Through Human Epidermis, 2005, Unpublished). Apart from the fact that there was a lack of supporting rat in vivo data, the following limitations were noted in the study:

- There was no standard reference compound tested to confirm reproducibility of the study results, as outlined in the OECD 428 guideline,
- No details on the dilution of the concentrate solution were provided. It was unclear whether an unlabeled compound was used in the dilution,

- The amount of residue retained on the skin may have been influenced, in part, to the prior freezing of the skin samples, rendering the skin more susceptible to accumulating residue,
- It is unclear if pure or formulated test substance was used, therefore, it is unclear whether the test product is representative of the proposed product,
- No details on the application method of the test substance to the skin samples were provided in the study report. As such, it is not known whether the application apparatus was analyzed for residues,
- No details as to the history of the human skin samples were provided in the study report (i.e. sex, extraction site, age of donor, etc.),
- It was unclear how the nominal doses, for undiluted formulated products, were derived,
- The results presented in table 4, for *stratum corneum*, are different than those presented in the appendix tables. If correction factors were used these were not detailed in the report,
- It is not mentioned if the test material was proven soluble in the receptor fluid. Receptor fluid ingredients were not stated,
- Although measured values did not fall below the LOQ, the values of LOQ and LOD were not provided,
- Rationale for testing formulated products (shampoo, body lotion and facial cream) was not provided, and
- Raw data and standard curves were not provided.