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STUDY TITLE

Mode of Action (MOA) for Nasal Tumors Induced by Methyl Isothiocyanate (MITC) in Sprague Dawley Rats

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Executive Summary

Methyl isothiocyanate (MITC) has been shown to induce nasal tumors in Sprague Dawley rats exposed to a high concentration (20 ppm) via whole body inhalation for 2 years. No tumors were observed at the lower exposure concentrations of 5 and 0.5 ppm, and no tumors were reported in a mouse inhalation carcinogenicity study with MITC. Non-neoplastic lesions in the nasal tissue have been reported in MITC acute repeat dose inhalation studies of various durations (1 day, 5 days, 4 weeks, 13 weeks, 52 weeks, and 104 weeks) in rats. In a mechanistic study, inhalation of 20 ppm MITC for 1 day, 5 days, or 4 weeks (5 days/week) induced nasal lesions consisting of acute/subacute inflammation, epithelial cell degeneration/necrosis, epithelial cell proliferation (epithelial hyperplasia, increased DNA synthesis detected by nuclear incorporation of BrdU, and regeneration), olfactory sensory neuron apoptosis, and olfactory sensory neuron loss resulting in atrophy/loss of olfactory epithelium and/or respiratory cell metaplasia. To understand the plausible mode of action (MOA) for rat nasal tumorgenicity, inhalation toxicity data were examined using the framework recommended by the International Programme on Chemical Safety (IPCS). The proposed nasal tumor MOA for MITC includes the following key events: 1) direct cytotoxicity of MITC in the nasal mucosa (e.g., degeneration, necrosis/apoptosis) because of its irritating properties; 2) cell proliferation to compensate for MITC-induced cytotoxicity; 3) onset and persistence of squamous cell metaplasia as an adaptive response to the cytotoxic insult; and 4) development of tumors, primarily squamous cell carcinomas. The data in support of the key events are discussed, along with possible alternative MOAs, and the human relevance of the proposed MOA. This analysis indicated confidence in the proposed MOA for MITC-induced rat nasal tumors and further informed that the tumorigenicity of MITC has thresholds and it is not likely to pose a carcinogenic risk to humans under normal use patterns and corresponding exposure levels.

Introduction: MITC Carcinogenicity in Rodents

MITC has been tested for carcinogenicity by the inhalation route in mice and rats. Chronic whole-body exposure (6 hour/day, 5 day/week for 104 weeks) to MITC at 20 ppm resulted in a statistically significant increase in the incidence of nasal tumors in male (Table 1) and female (Table 2) Sprague Dawley rats compared to controls [redacted], 2015a; summarized by EPA, 2018). Nasal tumors were not observed at the lower exposure concentrations of 0.5 and 5 ppm. Additionally, no increase in tumor incidence was reported in a 78-week carcinogenicity study in CD-1 mice with MITC concentrations of 0, 1, 5, and 15 ppm ([redacted] 2015b). There was also no evidence for MITC carcinogenicity even in mice or rats when administered via drinking water for 2 years (NRA, 1997). Therefore, the carcinogenicity of MITC is dose-dependent, shows a clear threshold below which no tumors are seen, and is limited to the portal of entry for the inhalation route in a species-specific manner.

Table 1. Nasal tumor incidence[a] in male rats from the 2-year MITC inhalation study

Dose (ppm)	0	0.5	5	20
Adenomas	0/60	0/60	0/60	3/60
(%)	(0)	(0)	(0)	(5)
(p-value)	0.0150*	1.0000	1.0000	0.1219
Sebaceous cell adenomas	1/60	1/60	0/60	0/60
(%)	(2)	(2)	(0)	(0)
(p-value)	0.1872	0.7521	1.0000	1.0000
Papillomas	0/60	0/60	0/60	6/60
(%)	(0)	(0)	(0)	(10)
(p-value)	0.0002**	1.0000	1.0000	0.0137*
Squamous cell papillomas	0/60	0/60	0/60	1/60
(%)	(0)	(0)	(0)	(2)
(p-value)	0.2500	1.0000	1.0000	0.5000
Anaplastic carcinomas	0/60	0/60	0/60	2/60
(%)	(0)	(0)	(0)	(3)
(p-value)	0.0617	1.0000	1.0000	0.2479
Squamous cell carcinomas	0/60	0/60	0/60	15/60
(%)	(0)	(0)	(0)	(25)
(p-value)	0.0000**	1.0000	1.0000	0.0000**
Carcinosarcomas	0/60	0/60	0/60	1/60
(%)	(0)	(0)	(0)	(2)
(p-value)	0.2500	1.0000	1.0000	0.5000
All nasal carcinomas combined	0/60	0/60	0/60	17[b]/60
(%)	(0)	(0)	(0)	(28)
(p-value)	0.0000**	1.0000	1.0000	0.0000**
All nasal tumors combined	1/60	1/60	0/60	25[b]/60
(%)	(2)	(2)	(0)	(42)
(p-value)	0.0000**	0.7521	1.0000	0.0000**

Data were extracted from the EPA (2018) CARC report on MITC.
[a] Number of tumor bearing animals/number of animals examined, excluding those that died or were sacrificed before week 52.
[b] Three animals in the 20 ppm dose group had multiple tumor types. Data for all combined nasal carcinomas was based on the animal count rather than tumor quantity; if an animal had multiple tumor sites, it was only counted once.
Significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level; * $p < 0.05$, ** $p < 0.01$.

Table 2. Nasal tumor incidence[a] in female rats from the 2-year MITC inhalation study

Dose (ppm)	0	0.5	5	20
Anaplastic carcinomas	0/60	0/60	0/60	2/60
(%)	(0)	(0)	(0)	(25)
(p-value)	0.06172	1.00000	1.00000	0.24790
Squamous cell carcinomas	0/60	0/60	0/60	15/60
(%)	(0)	(0)	(0)	(25)
(p-value)	0.00000**	1.00000	1.00000	0.00001**
All nasal carcinomas combined	0/60	0/60	0/60	16[b]/60
(%)	(0)	(0)	(0)	(27)
(p-value)	0.00000**	1.00000	1.00000	0.00000**

Data were extracted from the EPA (2018) CARC report on MITC.
[a] Number of tumor bearing animals/number of animals examined, excluding those that died or were sacrificed before week 52.
[b] One animal in the 20 ppm dose group had both an anaplastic carcinoma and a squamous cell carcinoma.
Significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level; ** $p < 0.01$.

The highest concentration of 20 ppm used in the carcinogenicity study in rats resulted in a significant decrement (compared to controls) both in absolute body weight (34% in males and 20% in females) and body weight gain (47% in males and 27% in females). Although this appeared to exceed a maximum tolerated dose (MTD), the argument for this was not accepted by the U.S. Environmental Protection Agency (EPA), and the study was deemed acceptable. It is noted that in the European Union (EU) review, the Rapporteur Member State (RMS) concluded that the highest dose exceeded the MTD (RMS Belgium, 2021). EPA's Cancer Assessment Review Committee (CARC) reviewed the available data to evaluate the carcinogenic potential of MITC via the inhalation route and concluded that the nasal tumors in male and female rats were treatment-related, and that there was no evidence of carcinogenicity in male or female mice (EPA, 2018). As a result of this evaluation, the CARC classified MITC via the inhalation route as "Likely to be Carcinogenic to Humans" based on treatment-related nasal tumors in both sexes in the rat. The [redacted] ([redacted] submitted a position paper to the EPA CARC, proposing that the highest concentrations in the rat inhalation chronic toxicity/carcinogenicity and mouse inhalation carcinogenicity studies exceeded the MTD based on the significantly reduced body weights in the high-dose animals, and therefore, would not be relevant for risk assessment ([redacted] 2017). However, the CARC concluded that the high test concentration was adequate and not excessively toxic for evaluating carcinogenicity" based on, among other points, no significant differences in early mortality with the high-dose groups (EPA, 2018). Therefore, the EPA's opinion on the carcinogenicity of MITC has remained the same since the 2018 CARC report.

To address a MOA for MITC nasal tumor formation, the [redacted] recently undertook a study to examine the potential MOA for the Sprague Dawley rat nasal tumors ([redacted] 2020). Only male Sprague Dawley rats were used in this study since both sexes responded similarly to tumor induction in the carcinogenicity study. The MOA framework described below was based on the data collected from male rats from various inhalation toxicities on MITC, including the recent [redacted] (2020) study.

The MOA presented below utilizes the MOA/human relevance framework that was developed by EPA and Health Canada (Meek et al., 2003; Cohen et al., 2004; Seed et al., 2005) and further elaborated by the IPCS (Sonich-Mullin et al., 2001; Boobis et al., 2006; 2008), which was incorporated into the EPA Cancer Guidelines (2005).

Postulated MOA for Rat Nasal Tumors Induced by MITC

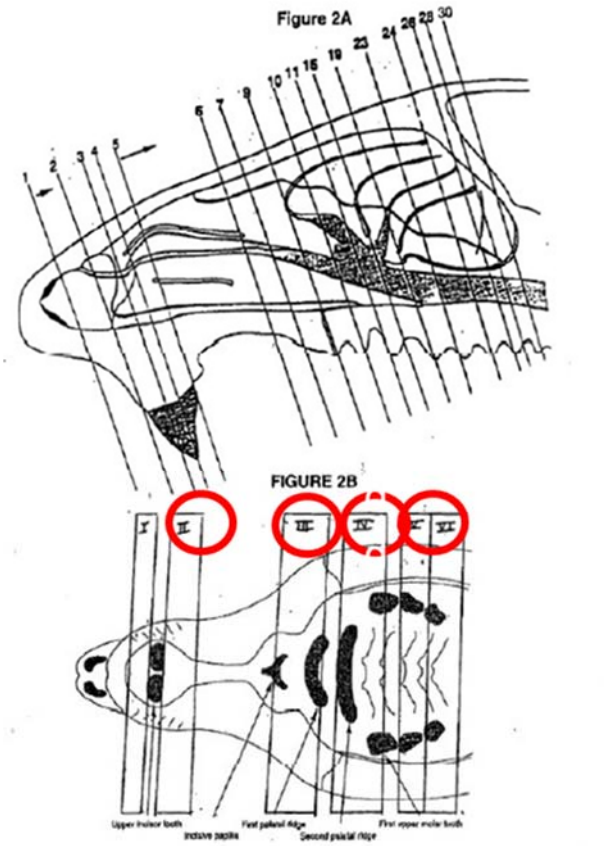
The postulated MOA for rat nasal tumors involves cytotoxicity occurring in the nasal passages once a threshold exposure concentration is exceeded. Cytotoxicity is the necessary factor in inducing tissue dysfunction (chronic inflammation), and leads to a consequent regenerative cell proliferation, hyperplasia, and squamous metaplasia. Increased cell proliferation leads to an increased opportunity for spontaneous errors to occur during DNA replication, increasing the risk of cellular transformation leading to tumors after MITC exposure (Moolgavkar and Knudson, 1981; Greenfield et al., 1984; Cohen and Ellwein, 1990; 1991; Tomasetti and Vogelstein, 2015; Tomasetti et al., 2017).

The available toxicity data on MITC reviewed in this paper supports the MITC-induced cytotoxicity with consequent regenerative cell proliferation as the MOA for the induction of the nasal tumors in rats. Since each of the key events in the MOA sequence is predicted to show clear thresholds before they trigger the subsequent event, this MOA demonstrated a threshold exposure concentration, below which tumorigenesis will not develop. This sequence of events for cytotoxicity and consequent regenerative increased cell proliferation has been widely demonstrated for a variety of chemicals involving various tissues in either rats and/or mice (see below) and has been accepted for the regulatory decision making on several of these chemicals. For all of these, the dose response involves a threshold, so that the risk assessment for the carcinogenicity also involves a threshold (see below for examples).

For most of the studies, the histopathology data on nasal passages of rats exposed by inhalation to MITC involved collection of six sections of the nasal cavities from each animal, except in the [redacted] (2020) study which collected four sections of the nasal cavity of each animal. The correspondence between the two methods is illustrated in Figure 1 below.

Figure 1. Correspondence between nasal sections collected for MITC inhalation studies in the rat.

The 4 red circles represent the 4 sections in [redacted] (2020) study corresponding to the 6 sections taken in the other reviewed studies



Sections are taken from the rat according to the diagram – Level I at section #2, Level II at section #5, Level III at section #9, Level IV at section #11, Level V at section #23 and Level VI at section #28

from: Toxicologic Pathology Volume 22, Number 4, 1994 p 355

Key Events for the MOA for the Formation of Nasal Tumors in Rats

Exposure concentration dependent non-neoplastic lesions in the nasal tissue have been reported in all MITC repeat dose inhalation studies in rats. These findings include epithelial cell and olfactory sensory neuron necrosis/degeneration, inflammation, and subsequent epithelial cell hyperplasia. These findings have been demonstrated across multiple rat studies. The key events for the nasal tumors induced in rats by MITC are the following: 1) direct cytotoxicity of MITC in the nasal mucosa (degeneration, necrosis/apoptosis); 2) consequent increased cell proliferation to compensate for the MITC-induced cytotoxicity; 3) onset and persistence of squamous cell metaplasia as an adaptive response to the cytotoxic insult; and 4) development of tumors, primarily squamous cell carcinomas.

Key Event 1: Direct Cytotoxicity of MITC in the Nasal Mucosa

Several studies of MITC inhalation in rats support Key Event 1. The most prominent tissue damage has been observed in the most forward of the respiratory epithelial-lined nasal passages (just distal to the normal squamous epithelium) of rats following inhalation exposure to MITC, where the highest exposure to MITC is expected to occur. The parts of the nasal passages more distal from the point of entry showed progressively less severe lesions. This indicates the importance of the portal of entry irritation and cytotoxicity effects of MITC. Observations of cytotoxicity included nasal epithelial degeneration, necrosis, and/or acute inflammation, and were consistently reported in the rat studies as early as one day post-inhalation exposure ([redacted] 2020; [redacted] 2011). These changes persisted to 104 weeks of inhalation exposure ([redacted] 2015a) as shown in Table 3 below.

Table 3. Microscopic observations of cytotoxicity for Key Event 1 (nasal epithelial degeneration, necrosis, and inflammation) in male rats from MITC inhalation studies

Dose (ppm)	Cytotoxicity observation	Exposure duration						
		1 Day						
		Evaluated Day 1[a/b]	Evaluated after recovery on Day 15[b]	5 Days[a]	4 Weeks[a/c]	13 Weeks[d]	52 Weeks[e]	104 Weeks[e]
0.5	Degeneration Necrosis Acute inflammation	No[a]/—[b] No[a]/—[b] No[a]/—[b]	—	No No No	No[a]/—[c] No[a]/—[c] No[a]/—[c]	—	No No No[f]	No[g] No No
1	Degeneration Necrosis Acute inflammation	—	—	—	—	No No[h] No	—	—
5	Degeneration Necrosis Acute inflammation	No[a]/—[b] No[a]/—[b] **Yes[a,i]**/—[b]	—	No No **Yes[j]**	No[a]/No[c] No[a]/No[c] **Yes[a,k]**/No[c]	No No[l] No	**Yes[m]** No No	**Yes[n]** No No
15	Degeneration Necrosis Acute inflammation	—	—	—	—	No **Yes[o]** **Yes[p]**	—	—
20	Degeneration Necrosis Acute inflammation	**Yes[a,q]**/No[b,r] **Yes[a,q]**/**Yes[b,s]** **Yes[a,t]**/No[b]	No No No	**Yes[u]** **Yes[u]** **Yes[v]**	**Yes[a,w]**/No[c] **Yes[a,w]**/No[c] **Yes[a,x]**/**Yes[c,y]**	—	**Yes[z]** No No	**Yes[aa]** No No
40	Degeneration Necrosis Acute inflammation	—[a]/**Yes[b,bb]** —[a]/**Yes[b,cc]** —[a]/No[b]	**Yes[dd]** No No	—	—[a]/**Yes[c,ee]** —[a]/No[c] —[a]/**Yes[c,ff]**	—	—	—
80	Degeneration Necrosis Acute inflammation	—[a]/**Yes[b,gg]** —[a]/**Yes[b,hh]** —[a]/**Yes[b,ii]**	**Yes[jj]** No No	—	—	—	—	—

—, Not tested in the study; "No", incidence was not increased compared to 0 ppm control or incidence was only 1 animal in the group; "Yes", increased incidence compared to the 0 ppm control group.

[a] [redacted] 2020; degeneration and necrosis reported together as a microscopic finding.

[b] [redacted] 2011; [c] [redacted] 2013; [d] [redacted] 2012; [e] [redacted] 2015a; [f] In 1/9 animals (mild) compared to 0/7 controls.

[g] In olfactory epithelium of 1/14 (moderate) animals compared to 0/14 in control group.

[h] In olfactory epithelium of 1/10 animals (minimal) compared to 0/10 in control group.

[i] In 7/8 animals (minimal) compared to 0/8 in control group.

[j] In 8/8 animals (minimal and mild) compared to 0/8 in control group.

[k] In 4/8 or 5/8 animals (depending on nasal section; minimal) compared to 0/8 in control group.

[l] In olfactory epithelium of 1/10 animals (minimal) compared to 0/10 in control group.

[m] In olfactory epithelium of 8/9 animals (minimal to moderate) compared to 0/7 in control group.

[n] In olfactory epithelium of 10/14 (minimal to moderate) animals and in respiratory epithelium of 1/14 (mild) animals compared to 0/14 in control group, respectively.

[o] In olfactory epithelium of 6/10 to 9/10 animals (depending on nasal level; minimal and mild) compared to 0/10 in control group.

[p] In 2/10 to 10/10 animals (minimal to moderate) compared to 0/10 to 2/10 in control group.

[q] Degeneration/necrosis in 5/8 animals (minimal and mild) compared to 0/8 in control group.

[r] In transitional epithelium of 1/6 animals (minimal) compared to 0/6 in control group.

[s] In olfactory epithelium of 1/6 to 2/6 animals (depending on nasal level; minimal) compared to 0/6 in control group.

[t] In 8/8 animals (minimal and mild) compared to 0/8 in control group.

[u] Degeneration/necrosis in 6/8 animals (mild and moderate) compared to 0/8 in control group.

[v] In 8/8 animals (minimal to marked) compared to 0/8 in control group.

[w] Degeneration/necrosis in 8/8 animals (minimal to marked) compared to 0/8 in control group.
[x] In 7/7 or 8/8 animals (depending on nasal section; mild to severe) compared to 0/8 in control group.
[y] In 1/5 or 5/5 animals (depending on nasal level; minimal) compared to 0/5 or 1/5 in control group.
[z] In olfactory epithelium of 6/7 (minimal to moderate) animals compared to 0/7 in control group.
[aa] In olfactory epithelium of 5/14 (minimal to severe) animals compared to 0/14 in control group.
[bb] In transitional epithelium of 6/6 animals (minimal to moderate) and in respiratory epithelium of 1/6 animals (minimal) compared to 0/6 in control group.
[cc] In olfactory epithelium of 1/6 to 6/6 animals (depending on nasal level; minimal to severe) compared to 0/6 in control group.
[dd] In olfactory epithelium of 1/6 to 6/6 animals (depending on nasal level; minimal and mild) compared to 0/5 in control group.
[ee] In transitional epithelium of 2/5 animals (minimal and mild) compared to 0/5 in control group.
[ff] In 5/5 animals (minimal to moderate) compared to 0/5 or 1/5 in control group.
[gg] In transitional epithelium of 6/6 animals (mild to severe) and in respiratory epithelium of 3/6 to 5/6 animals (depending on nasal level; mild and moderate) compared to 0/6 in control group, respectively.
[hh] In olfactory epithelium of 4/6 to 6/6 animals (depending on nasal level; minimal to severe) compared to 0/6 in control group.
[ii] In 3/6 to 4/6 animals (depending on nasal level; minimal and mild) compared to 0/6 in control group.
[jj] In olfactory epithelium of 2/6 to 6/6 animals (depending on nasal level; minimal and mild) compared to 0/5 in control group

In [redacted] (2020), histopathologic and morphometric examinations were conducted in male Sprague Dawley rats (8/group) exposed by inhalation to 0, 0.5, 5, or 20 ppm MITC, for 6 hours/day for one day, five days, or four weeks (5 days/week for 28 days). Additional groups were included for recovery evaluations four weeks after the 4-week exposure ended. The early timepoint findings, reported by [redacted] (2020) supports Key Event 1 in establishing cytotoxicity as being the first triggering event required to begin the process towards neoplastic development. The [redacted] (2020) study demonstrated the following findings at the 20 ppm MITC exposure: histopathologic changes in the nasal airways consisting of acute/subacute inflammation, epithelial degeneration and necrosis, olfactory sensory neuron apoptosis (after one day of exposure), and olfactory sensory neuron loss resulting in atrophy/loss of olfactory epithelium and respiratory metaplasia (after four weeks of exposure). Exposure to 5 ppm MITC resulted in less severe cytotoxicity in the respiratory epithelium, minimal to mild nasal tissue inflammation, no histopathological abnormality of olfactory epithelium (see Table 3), and as discussed below, no evidence of squamous cell metaplasia (and Table 7), unlike the findings present in 20 ppm MITC-exposed rats. Animals exposed to 0.5 ppm MITC had no exposure-related histopathology.

The study by [redacted] (2011) also demonstrated signs of cytotoxicity following a single, acute exposure to MITC on study day one at doses ≥ 20 ppm (see Table 3). The findings from both of the acute inhalation

studies (2011; 2020) demonstrate that high doses of MITC can induce the initial key event of cytotoxicity in the nasal tissue, and that it occurs as early as one day after MITC inhalation exposure in rats (the earliest time examined).

A 4-week inhalation study involving exposures to doses of 5, 20, and 40 ppm MITC for 6 hours/day, 5 days/week in rats demonstrated nasal lesions indicative of irritant and cytotoxic effects of MITC including acute inflammation at the doses of 20 and 40 ppm and epithelial lining degeneration at 40 ppm (2013). Similar findings were observed in rats from the (2020) study at the 4-week timepoint as shown in Table 3, but with inflammation of nasal tissue occurring at ≥ 5 ppm and nasal epithelial degeneration necrosis reported at 20 ppm.

With a more prolonged MITC exposure of 13-weeks, microscopic lesions in the nasal mucosa indicative of cytotoxicity and inflammation were observed in rats at 15 ppm (2012). Only minimal to moderate grade degeneration was observed at 5 ppm. Furthermore, severe degeneration was observed in male rats from the 20-ppm group at 104 weeks suggesting that the extent of cytotoxicity is exposure-duration dependent. These non-neoplastic findings were accompanied by atrophy of Bowman's glands, olfactory nerve bundles and olfactory epithelium in the 5- and 20-ppm groups. The effects observed in the nasal olfactory epithelium were responsible for atrophy in the olfactory bulb, which was observed at a high incidence and severity in the 20-ppm group.

Overall, the cytotoxicity observations from these studies are clearly dose- and exposure time-dependent in rat nasal tissue. Altogether, these findings support Key Event 1 and indicate that the irritant and cytotoxic effects of MITC persist in the nasal tissue with repeated and prolonged exposures with the severity of the effects dependent on the exposure level.

Key Event 2: Cell Proliferation to Compensate for MITC-Induced Cytotoxicity

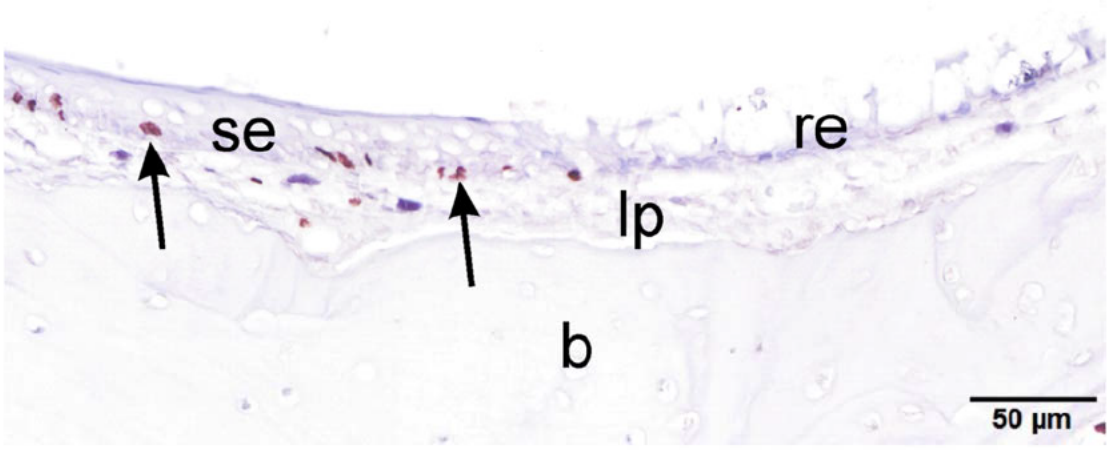
As described above for Key Event 1, prolonged exposure to MITC induces sustained, concentration-dependent cytotoxicity in the nasal tissue of rodents. The local tissue response of chronic irritation and cytotoxicity is postulated to include increased cell proliferation in the nasal tissue to compensate for the cytotoxicity and represents Key Event 2. This is the usual tissue response to cell death since the cellularity of the tissue needs to be replaced to maintain the biologic function of the tissue.

Evidence for Key Event 2 comes directly from the [redacted] (2020) study, in which rats exposed to 5 ppm and 20 ppm MITC were observed to have increased nasal cavity cell proliferation compared to control animals. This was demonstrated through both microscopic observations of nasal cavity epithelial hyperplasia and measurements of DNA synthesis detected by nuclear incorporation of bromodeoxyuridine (BrdU) in nasal tissue (Figure 2). BrdU incorporation is a common method used to assess DNA replication (Wood et al., 2015).

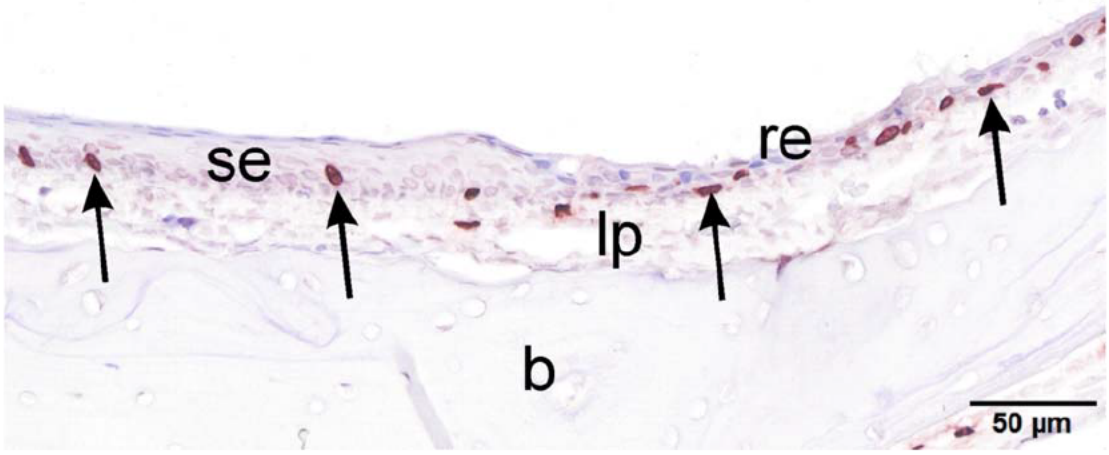
Figure 2.

A. Nasal tissue from a control rat treated for one day is an inhalation chamber for 6 hours at 0 mg/m^3 showing immunohistochemical labeling for BrdU indicating cell replication (positive cells stain brown, a few indicated by arrows). "se" is for the squamous epithelium, and "re", the respiratory epithelium. Note that the number of labeled cells is significantly greater in the normal squamous epithelium than in the normal respiratory epithelium, which is typical of these specific types of tissues (Johnson et al., 1990).
B. Nasal tissue from a rat on day one after treatment by inhalation for 6 hours with 20 mg/m^3 MITC, showing similar labeling of the squamous epithelium (se) as in controls but with a markedly increased labeling of the respiratory epithelium (re). Note that the respiratory epithelium shows evidence of severe toxicity, including loss of mucin-containing goblet cells, necrosis, and cellular degenerative changes. "lp", lamina propria; "b" bone.
(Photographs provided by [redacted], who performed the study reported in [redacted] 2020.)

A



B



Quantitative BrdU measurements from this study showed increased DNA synthesis in the transitional epithelium lining of the maxilla turbinates at 5 ppm and 20 ppm. As shown in Table 4, the highest BrdU incorporation epithelial cells were observed after one day of MITC exposure at these dose levels. However, cell proliferation was still found to be significantly increased compared to controls at the 5-day and 4-week timepoints at both 5 and 20 ppm, *albeit* at significantly lower levels compared to their respective 1-day levels. Since hyperplasia is present, there is not only an increase in the rate of proliferation, but the number of cells present has also increased, which would be a multiplicative effect on the rate. Rate is defined as the number of labeled cells over the total number of cells. Since the total number of cells is increased (hyperplasia) and the rate is increased, the number of labeled cells is markedly increased in these tissues. The critical parameter is the number of DNA replications, not the rate. Thus, the increased DNA replication likely persisted throughout the entire time of the 2-year bioassay, as a result of MITC-induced cytotoxicity. The initial increases in rate followed by a return to control levels but with increased cell number is similar to what has been observed in various other examples in animal models, such as liver tumorigenesis by CAR activators (Elcombe et al., 2014; Yamada et al., 2021) and PPARα activators (Corton et al., 2014; 2018).

$$Rate = \frac{\text{Number of Labeled Cells}}{\text{Total Number of Cells}}$$

$$Number\ of\ Labeled\ Cells = Rate\ X\ Total\ Number\ of\ Cells$$

The recovery groups from this study (i.e., four weeks of exposure followed by a 4-week period of no MITC exposure) had statistically similar BrdU-labeled nuclei among all the exposure groups including untreated controls. This indicates that MITC-induced increases in DNA synthesis and cellular replication in the epithelium lining of the proximal nasal airways is not persistent following stoppage of exposure. Again, this is a typical cellular response as the tissue is repaired and no longer needs to have increased cell proliferation to replenish damaged cells.

Table 4. Unit Length Labelling Index of BrdU-labeled nuclei in transitional epithelium lining the maxilloturbinates (T1 nasal section) of male rats

MITC dose (ppm)	Approximate mean BrdU-labeled nuclei/mm basal lamina length (mean ± SEM)			
	1 Day exposure	5 Days exposure	4 Weeks exposure	Recovery (4 weeks)
0	4.2 ± 0.63	3.2 ± 0.47	2.3 ± 0.24#	1.6 ± 0.3
0.5	3.0 ± 0.50	2.4 ± 0.45	3.2 ± 0.38	1.3 ± 0.3
5	33.5 ± 3.46*	12.0 ± 0.80*,#	9.7 ± 0.99*,#	1.3 ± 0.3
20	53.8 ± 1.88*	12.8 ± 1.88*,#	19.3 ± 3.31*,#	1.5 ± 0.2

* Statistically significant compared to respective control group at exposure day 1, 5, or 20; $p < 0.05$.
\# Statistically significant compared to 1-day respective dose group, $p < 0.05$

The histopathology data from animals exposed to 20 ppm MITC for one day in this study revealed minimal or mild epithelial cell hyperplasia of transitional and respiratory epithelium in the proximal and middle nasal cavity sections (see Table 5). These observations for the 20-ppm group increased in severity from minimal and mild after one day to moderate and marked after both 5 days and 4 weeks of exposure. Epithelial cell hyperplasia was also reported in the 5-ppm animals at each of the time points, and when compared to the 20-ppm concentration, the severity of the lesions was minimal or mild and only present in the proximal nasal airways. Observations of increased nasal epithelial hyperplasia and regeneration were also reported in other MITC inhalation studies in rats with a variety of exposure durations (4, 13, 52, and 104 weeks) ([redacted] 2012; 2013; 2015a). A summary of the MITC induced hyperplasia findings in nasal tissue across all the rat inhalation studies is presented in Table 5.

As shown in Table 5, hyperplastic changes in nasal tissue were reported in all the MITC inhalation studies in rats. While this finding was primarily associated with repeated exposure to MITC, [redacted] (2020) observed hyperplasia with doses ≥ 5 ppm following a single inhalation exposure to MITC. In the 4- and 13-week studies, increased incidences of nasal epithelial hyperplasia were observed in males at doses ≥ 5 ppm. However, after 104 weeks of MITC exposure, increased hyperplasia was reported only at 20 ppm in the males. Hyperplasia of the squamous epithelium was only increased in the 20-ppm group across all

summarized studies in Table 5. Therefore, increased regenerative cell proliferation appears to be dose- and exposure time-dependent in rat nasal tissue.

Table 5. Microscopic observations of increased nasal epithelial hyperplasia incidence in male rats from MITC inhalation studies

Dose (ppm)	Exposure duration						
	1 day		5 Days[a]	4 Weeks[a/c]	13 Weeks[d]	52 Weeks[e]	104 Weeks[e]
	Evaluated Day 1[a/b]	Evaluated after recovery on Day 15[b]					
0.5	No[a]/—[b]	—	**Yes**[f]	No[a]/—[c]	—	No	No
1	—	—	—	—	No	—	—
5	**Yes**[a,g]/—[b]	—	**Yes**[h]	**Yes**[a,i]/No[c]	No	No	No
15	—	—	—	—	**Yes**[j]	—	—
20	**Yes**[a,k]/No[b]	No	**Yes**[l]	**Yes**[a,m]/**Yes**[c,n]	—	**Yes**[o]	**Yes**[p]
40	—[a]/No[b]	No	—	—[a]/**Yes**[c,q]	—	—	—
80	—[a] /No[b]	No	—	—	—	—	—

—, Not tested in the study; "No", incidence was not increased compared to 0 ppm control or incidence was only 1 animal in the group; "Yes", increased incidence compared to the 0 ppm control group.

[a] [redacted] 2020

[b] [redacted] 2011

[c] [redacted] 2013

[d] [redacted] 2012

[e] [redacted] 2015a

[f] 2/8 animals with hyperplasia of transitional and respiratory epithelium (minimal) compared to 0/8 in control group.

[g] 7/8 animals with hyperplasia of transitional and respiratory epithelium (minimal and mild) compared to 0/8 in control group.

[h] 8/8 animals with hyperplasia of transitional and respiratory epithelium (minimal and mild) compared to 0/8 in control group.

i 4/8 or 6/8 animals with hyperplasia of transitional and respiratory epithelium (depending on nasal section; minimal and mild) compared to 0/8 in control group.

[j] 2/10 (minimal) animals with squamous epithelium hyperplasia compared to 0/10 in control group.

[k] 7/8 or 8/8 animals with hyperplasia of transitional and respiratory epithelium (depending on nasal section; minimal and mild) compared to 0/8 in control group.

[l] 8/8 animals with hyperplasia of transitional and respiratory epithelium (depending on nasal section; minimal to marked) compared to 0/8 in control group.

[m] 7/8 or 8/8 animals with hyperplasia of transitional and respiratory epithelium (depending on nasal section; mild to marked) compared to 0/8 in control group.

[n] 5/5 animals with squamous epithelium hyperplasia (minimal and mild) compared to 0/5 in control group; 3/5 to 5/5 animals (depending on nasal level; minimal to moderate) with respiratory epithelium hyperplasia compared to 0/5 in control group.

[o] 4/10 animals with squamous epithelium hyperplasia (minimal) compared to 1/10 in control group.

[p] 12/14 animals with squamous epithelium hyperplasia (minimal and mild) compared to 5/14 in control group; 14/14 animals with respiratory epithelium/mucous cell hyperplasia (minimal to severe) compared to 7/14 in control group.

[q] 5/5 animals with squamous epithelium hyperplasia (moderate to severe) compared to 0/5 in control group; 3/5 to 5/5 animals (depending on nasal level; minimal to moderate) with respiratory epithelium hyperplasia compared to 0/5 in control group.

The local tissue response of regenerative cell proliferation discussed above is expected to persist with continued exposures to MITC, and this is supported by the data from [redacted] (2020). Evidence for MITC-induced sustained hyperplasia has been demonstrated through the consistent observation of increased

hyperplasia in nasal epithelial cells across the sub-chronic and chronic rat inhalation studies reviewed for this paper (2012; 2015a). This is summarized in Table 5, which shows the persistence of increased hyperplasia in the nasal epithelium in male rats following 52- and 104-week exposures to MITC, and especially at the high doses. Further, squamous epithelial hyperplasia, specifically, was increased at 20 ppm across all the summarized timepoints included in Table 8. Hyperplasia in this specific cell type aligns with the statistically significant increase in squamous cell carcinomas reported at this dose level (see Tables 1 and 2), suggesting that hyperplasia is a critical pre-neoplastic lesion, as typically observed in epithelial tissue carcinogenesis (Wood et al., 2015).

As discussed before, the (2020) study demonstrated increased cell proliferation following MITC inhalation exposure by using a quantitative cell proliferation marker (BrdU). While the longest timepoint was only 4 weeks, the findings for the 5-ppm and 20-ppm groups quantitatively demonstrated that increased cell proliferation was sustained throughout the course of the study. Although proliferation rates associated with the 5-ppm group returned to control levels, the 20-ppm exposure group appeared to have plateaued at the end of this study (see Table 4). However, since hyperplasia continued, there continued to be increased cell proliferation at these doses based on the increased cell number, even with the labeling index at 5 ppm being at control levels.

Additionally, regeneration in nasal epithelium was noted in the histopathologic findings from the 4- and 13-week rat MITC inhalation studies. As shown in Table 6, male rats had increased nasal epithelial regeneration at 20 ppm and 40 ppm after four weeks exposure. After 13 weeks, this was reported at the highest tested dose of 15 ppm. The observations indicated dose- and time-dependent association of MITC exposure in nasal epithelial regeneration.

Table 6. Microscopic observations of increased nasal epithelial regeneration incidence in male rats from MITC inhalation studies

Dose (ppm)	Exposure Duration	
	4 Weeks[a]	13 Weeks[b]
0.5	—	—
1	—	No[c]
5	No	No[d]
15	—	Yes[e]
20	Yes[f]	—
40	Yes[g]	—

—, Not tested in the study; "No", incidence was not increased compared to 0 ppm control or incidence was only 1 animal in the group; "Yes", increased incidence compared to the 0 ppm control group.

[a] [redacted] 2013

[b] [redacted] 2012

[c] 1/10 animals with olfactory epithelium regeneration (moderate) compared to 0/10 in control group.

[d] 1/10 animals with olfactory epithelium regeneration (mild) compared to 0/10 in control group.

[e] 6/10 to 10/10 animals (depending on nasal level) with olfactory epithelium regeneration (mild to severe) compared to 0/10 in control group; 3/10 to 10/10 animals (depending on nasal level) with respiratory epithelium regeneration (minimal and mild) compared to 0/10 in control group; 8/10 animals with transitional epithelium regeneration (minimal and mild) compared to 0/10 in control group.

[f] 1/5 to 5/5 animals (depending on nasal level) with olfactory epithelium regeneration (mild and moderate) compared to 0/5 in control group; 2/5 to 5/5 animals (depending on nasal level) with respiratory epithelium regeneration (minimal to moderate) compared to 0/5 in control group.

[g] 3/5 animals with squamous epithelium regeneration (minimal and mild) compared to 0/5 in control group; 5/5 animals with respiratory epithelium regeneration (minimal to moderate) compared to 0/5 in control group; 5/5 animals with olfactory epithelium regeneration (minimal and mild) compared to 0/5 in control group.

Overall, these finding support Key Event 2 and indicate that cell regeneration and increased cell proliferation effects of MITC occur in the nasal cavity tissue with repeated exposures and aligned with the observations of persistent cytotoxicity. These findings also indicate the presence of sustained cell proliferation in the nasal tissue with repeated exposures for the entire time of MITC exposure, up to two years. Results of the recovery studies further indicate that MITC-induced cell proliferation does not persist following cessation of exposure.

Key Event 3: Onset and Persistence of Squamous Metaplasia as an Adaptive Response to the Cytotoxic Insult

Evidence for Key Event 3 is supported by the observation of increased squamous cell metaplasia in rats following repeat dose exposures, as summarized in Table 7. This finding was reported in rats exposed to the high dose of 20 ppm that induced nasal tumors in the rat carcinogenicity study.

Table 7. Microscopic observations of increased squamous cell metaplasia incidence in male rats from MITC inhalation studies

Dose (ppm)	Exposure duration						
	1 day		5 Days[a]	4 Weeks[a/c]	13 Weeks[d]	52 Weeks[e]	104 Weeks[e]
	Evaluated Day 1[a/b]	Evaluated after recovery on Day 15[b]					
0.5	No[a]/—[b]	—	No	No[a]/—[c]	—	No[f]	No[g]
1	—	—	—	—	No	—	—
5	No[a]/—[b]	—	No	No[a]/No[c,h]	No	No[i]	(Yes)[j]
15	—	—	—	—	**Yes**[k]	—	—
20	No[a] No[b]	No	**Yes**[l]	**Yes**[a,m]/**Yes**[c,n]	—	**Yes**[o]	**Yes**[p]

—, Not tested in the study; "No", incidence was not increased compared to 0 ppm control or incidence was only 1 animal in the group; "**Yes**", increased incidence compared to the 0 ppm control group.

[a] [redacted] 2020

[b] [redacted] 2011

[c] [redacted] 2013

[d] [redacted] 2012

[e] [redacted] 2015a

[f] 7/9 animals with squamous metaplasia (minimal and mild) compared to 4/7 (minimal) in control group.

[g] 1/14 or 3/14 animals (depending on nasal level) with squamous metaplasia (minimal and mild) compared to 1/14 (minimal) in control group.

[h] 1/5 animals with squamous metaplasia (minimal) compared to 0/5 in control group.

[I] 7/9 animals with squamous metaplasia (minimal to moderate) compared to 4/7 (minimal) in control group.

[j] Low severity effect; 10/14 animals with minimal and mild squamous metaplasia compared to 1/14 animals with minimal severity in control group.

[k] 10/10 animals with squamous metaplasia (minimal to moderate) compared to 0/10 in control group.

[l] 6/8 animals with squamous metaplasia (minimal to moderate) compared to 0/8 in control group.

[m] 8/8 animals with squamous metaplasia (minimal to severe) compared to 0/8 in control group.

[n] 5/5 animals with squamous metaplasia (minimal and mild) compared to 0/5 in control group.

[o] 6/7 animals with squamous metaplasia (moderate and severe) compared to 4/7 (minimal) in control group.

[p] 14/14 animals with squamous metaplasia (minimal to severe) compared to 1/14 (minimal) in control group.

As shown in Table 7, increased incidence and severity of squamous cell metaplasia was observed only with repeated exposures to high doses of MITC. While some animal had this finding at lower doses with exposure durations of 4, 13, and 52 weeks, in those cases the incidence was not much greater than in control

groups and/or with only minimal or mild severity. Further, after 104 weeks of exposure, this finding was present at all tested doses but with increasing incidence and severity as the dose increased (see Table 7). Therefore, the onset and persistence of squamous cell metaplasia supports Key Event 3, including the incidence and severity of this change of the 20-ppm exposure level exceeds the threshold for triggering the next event, the eventual development of tumors, in rats at this exposure level.

Squamous metaplasia is the usual response of cytotoxicity of various columnar epithelia such as the respiratory epithelium of the nasal cavity, but also occurs in the respiratory tract epithelium (Renne et al., 2009) of the larynx, trachea, and bronchi, as well as in some other tissues, such as the uterine cervix (Malpica, 2014). This is a typical response to toxicity in these columnar epithelial tissues, with the squamous metaplasia persisting as long as the toxic insult persists.

It should be noted that squamous epithelium, whether normally present or metaplastic, has a greater rate of proliferation than the corresponding columnar epithelium, such as the respiratory epithelium of the nasal cavity (Johnson et al., 1990; Harkema et al., 2006) (See Figure 2). This adds to the proportion of increased cell proliferation, as there is not only an increase in rate, but there is an increase in the number of cells as evident by hyperplasia, as well as the increase in squamous epithelium versus respiratory columnar epithelium.

Key Event 4: Development of Tumors

As presented in Table 1, male rats develop nasal tumors following two years of exposure to 20 ppm MITC. Male rats from the 20-ppm group showed a significant trend for nasal adenomas ($p < 0.05$); pairwise comparisons were significant for squamous cell papillomas ($p < 0.05$) and squamous cell carcinomas ($p < 0.01$); and all nasal tumors combined (adenomas, papillomas, and carcinomas) were significantly increased

($p < 0.01$) by both trend and pairwise comparisons. Tumors occurred only at the 20-ppm dose level, corresponding to the dose with severe cytotoxicity, increased regenerative cell proliferation and hyperplasia, and squamous cell metaplasia. The onset and persistence of squamous cell metaplasia, at a dose level exceeding the necessary threshold, leads to the eventual development of tumors at the 20-ppm exposure level.

The MOA framework utilizes modified Bradford Hill (1965) criteria for evaluating causality and whether acceptable data is available to support the actual MOA (Sonich-Mullin et al., 2001). These criteria include dose response, temporality, strength, consistency, and specificity, and biologic plausibility and coherence. Consideration of alternative MOAs has been assessed as well as an evaluation of possible uncertainties, inconsistencies, and data gaps. An overall assessment is then made as well as an assessment of human relevance. Finally, a statement of confidence, analysis, and implications for human risk assessment is made.

Dose Response Relationships for the Key Events

For this MOA, there is an expectation of dose-related occurrence of key events such that lower doses induce precursor key events before the manifestation of the subsequent key event at higher doses along the pathway to tumorigenesis. In all the rat repeat-dose inhalation toxicity studies on MITC reviewed in this paper, the key events occurred in a dose-dependent manner. Table 8 presents the dose-response relationship for the key events in the MITC-induced nasal tumor MOA.

Temporality of the Key Events

The key events are expected to occur in the order in which they are proposed, where the key non-neoplastic changes included in Key Events 1-3 precede the appearance of tumors. For MITC, the key events necessary to demonstrate the rat-specific nasal tumor MOA are available from multiple inhalation exposure durations (1 day, 5 days, 4 weeks, 13 weeks, 1 year, and 2-year studies) and a temporal association of progression is demonstrated.

Table 8. Dose and temporal concordance for the key events for MITC-induced nasal tumors in male rats

Dose	Temporal association →																
		Key Events															
	Dose (ppm)	1: Cytotoxicity					2: Cell proliferation/ hyperplasia					3: Squamous cell metaplasia					4: Tumor development
		1 D	5 D	4 W	13 W	52 W	1 D	5 D	4 W	13 W	52 W	1 D	5 D	4 W	13 W	52 W	104 W
	0.5	N	N	N	-	N	N	N	N	-	N	N	N	N	-	N[a]	N
	1	-	-	-	N	-	-	-	-	N	-	-	-	-	-	-	-
	5	Y[b]	Y[b]	Y[b]	N	Y[b]	Y[c]	Y[c]	Y[c]	N	N	N	N	N	-	N[d]	N
	15	-	-	-	Y[e]	-	-	-	-	Y[f]	-	-	-	-	Y[g]	-	-
	20	Y[h]	Y[h]	Y[h]	-	Y[h]	Y[i]	Y[i]	Y[i]	-	Y[i]	N	Y[j]	Y[j]	-	Y[j]	Y[k]

-, Dose not tested in the study; **Y**, observed in >2 animals and with a higher incidence and/or severity than the control group.
[a] See Table 10, footnote f for incidence and severity.
[b] Inflammation at 1 day, 5 days, and 4 weeks ([redacted] 2020; [redacted] 2012; [redacted] 2013); degeneration at 52 weeks ([redacted] 2015a).
[c] Increased cell proliferation using BrdU labeling at 1 day, 5 days, and 4 weeks; minimal to mild hyperplasia of transitional/respiratory epithelium at 1 day, 5 days, and 4 weeks ([redacted] 2020).
[d] See Table 10, footnote i for incidence and severity.
[e] Necrosis and inflammation at 13 weeks ([redacted] 2012).
[f] Minimal squamous epithelium hyperplasia at 13 weeks ([redacted] 2012).
[g] Squamous cell metaplasia at 13 weeks ([redacted] 2012).
[h] Degeneration, necrosis, and/or inflammation at 1 day, 5 days, and 4 weeks ([redacted] 2020; [redacted] 2011; [redacted] 2013); degeneration only at 52 weeks ([redacted] 2015a).

[i] Increased cell proliferation using BrdU labeling at 1 day, 5 days, and 4 weeks ([redacted] 2020); hyperplasia of transitional/respiratory epithelium and/or squamous epithelium hyperplasia (ranging from minimal to marked) at 1 and 5 days, and at 4 and 52 weeks ([redacted] 2020; [redacted] 2013; [redacted] 2015a).
[j] Squamous cell metaplasia at 5 days and 4 weeks ([redacted] 2020) and at 52 weeks ([redacted] 2015a).
[k] First squamous cell carcinoma observed at week 39 ([redacted] 2015a).

Key Event 1 (cytotoxicity) has been observed in the nasal mucosa of rats with any exposure duration, from acute (1 day) to chronic (up to 2 years) exposures. One day is the earliest timepoint that has been examined. An acute inhalation study ([redacted] 2011) demonstrated that while cytotoxicity occurred following a single exposure to high levels of MITC, hyperplasia (Key Event 2) was not observed in those tissue sites one day or two weeks after the initial exposure. This indicates the temporal association between Key Event 1 and 2.

The temporality of Key Event 2 (cell proliferation) was also demonstrated in the [redacted] (2020) study via BrdU incorporation in epithelial cells, with statistically significant increases in cell proliferation reported compared to controls at the 1-day, 5-day, and 20-day timepoints at doses ≥ 5 ppm (see Table 4). This study also demonstrated a temporal association of hyperplasia findings in nasal sections, with 20-ppm group animals exhibiting only minimal-to-mild epithelial hyperplasia after 1 day, mild-to-moderate hyperplasia after 5 days and moderate-to-marked hyperplasia after 4 weeks. The increase in cell proliferation over a time course of exposures was qualitatively demonstrated across the various rat inhalation studies, such that increased nasal tissue hyperplasia was reported beginning after one day (see Table 5). Additionally, regeneration in nasal epithelium was noted in the histopathologic findings from the 4- and 13-week rat MITC inhalation studies (see Table 6).

Squamous cell metaplasia (Key Event 3) was not evident following a single exposure; however, this key event was evident following 5 days of exposure and persisting through other longer exposure durations (see Table 7).

Finally, Key Events 1-3 preceded the development of nasal tumors (first observed at week 39 in males; [redacted] 2015a). As shown in Table 8, there is a clear temporal association for the key events in the MITC-induced nasal tumor formation in which all key events preceded tumor formation.

Strength, Consistency, and Specificity of the Tumor Response with the Key Events

The weight of evidence linking the key events supports a MOA where cytotoxicity leads to chronic tissue damage followed by sustained increased cell proliferation and hyperplasia, squamous metaplasia, and ultimately tumor formation in the rats. The strength and consistency of association between exposure to high concentrations of MITC and the incidence of nasal tumors in rats is supported by the studies described above, which demonstrate a concordance of dose-response and temporal relationships across the key events. Strength addresses the magnitude at which key events in the proposed MOA are observed, whereas consistency addresses the repeatability of key events across the different studies.

Regarding Key Event 1, initial signs of nasal epithelial cytotoxicity (cell degeneration, necrosis, and acute inflammation) were observed following a single, 6-hour exposure to high doses of MITC (20-80 ppm) in the acute rat MITC exposure study by [redacted] (2011). This study demonstrated partial resolution of necrosis and acute inflammation in an evaluation two weeks after the acute inhalation exposure. While nasal tissue degeneration was still reported on study day 15, the incidence rates appear to trend downward (see Table 3). In [redacted] (2020), histopathologic examinations of the 20-ppm MITC group nasal airway tissue demonstrated acute/subacute inflammation, epithelial cell degeneration and necrosis, and olfactory sensory neuron apoptosis after one day of inhalation exposure in rats. There were statistically significant increases in the severity scores for acute/subacute inflammation on study day 5 and 28 compared to study day 1 as well as when compared to the controls. This was accompanied by a mixed inflammatory cell infiltrate composed of neutrophils with lower levels of lymphocytes, indicating a severe inflammatory response. In 6 of the 8 rats exposed to 20 ppm MITC, there was epithelial regeneration/necrosis of apical cells in both transitional and respiratory epithelium. Taken together, these observations demonstrate the

strength and the consistency of Key Event 1 as being the necessary initial step in the proposed MOA that must precede Key Event 2, increased regenerative cell proliferation and hyperplasia.

With respect to Key Event 2, [redacted] (2020) observed markedly increased cell proliferation in nasal epithelial cells in rats with 5 and 20 ppm MITC exposure compared to controls at timepoints up to four weeks (see Table 4). This was accompanied by increased severity of hyperplasia of transitional and respiratory epithelium in the proximal and middle nasal sections following 5 days and 4 weeks of exposure (see Table 5). As shown in Table 6, regeneration of nasal epithelial tissue was observed in the [redacted] (2013) 4-week MITC exposure study at the highest doses tested (20 and 40 ppm) and in the sub-chronic (13-week) study at the highest dose tested (15 ppm). The consistent observation of hyperplasia in the nasal epithelium at high MITC doses across the sub-chronic and chronic timepoints suggests that regenerative cell proliferation/hyperplasia in response to cytotoxicity in nasal epithelial cells is sustained with prolonged exposures to MITC. Additionally, as discussed above, the [redacted] (2020) study demonstrated regenerative cell proliferation throughout 28 days of MITC inhalation exposure using BrdU as a proliferation marker. While the longest timepoint was only 28 days in this study, the findings for the 20-ppm group quantitatively supports that increased cell proliferation was sustained throughout the course of studies, even though the data suggests that the rate was lower at later timepoints in the study (see Table 4). However, the presence of hyperplasia indicates that there is an increase in the cell number, which would mean that there is an increase in cell proliferation even if the rate of proliferation was the same as in controls. Overall, these data demonstrate the strength of Key Event 2 of being the next step in the proposed MOA, which occurs as a reparative mechanism following more prolonged MITC exposures. The increased cell proliferation is demonstrated both by the increase in cell proliferation rate (labeling index) as well as in the increased number of cells (hyperplasia), and furthermore, by the development of squamous metaplasia versus similar findings in the respiratory columnar epithelium.

The strength and specificity of Key Event 3 is supported by the observation of increased squamous cell metaplasia that shows an increased incidence in severity at the 20 ppm that clearly exceeds the threshold required to trigger the next downstream event, neoplasia. Squamous metaplasia of this specific cell type aligns with statistically significant increase in squamous cell carcinomas reported at this dose level (see Table 1) demonstrating the tumor response, and this key event occurs in the same cell type (squamous cell) and site of action (nasal epithelium).

Squamous cell metaplasia appears to be a good predictor of tumorigenesis, given that it was observed in most of the nasal levels of the 20-ppm group animals and in the same areas in which squamous cell carcinomas were observed in the chronic rat study ([redacted] 2015a). Squamous cell metaplasia was not reported for the 5-ppm dose groups, nor were squamous cell carcinomas, the primary tumor type in the [redacted] (2015a) study. Further, [redacted] (2020) reported increased squamous cell metaplasia after 5- and 28-days exposure in the 20-ppm group only.

Together these observations are supportive of Key Event 3 as being the transitional step between regenerative hyperplasia and the development of nasal tumors in the proposed MOA.

The development of nasal tumors (Key Event 4) was only observed at 20 ppm in the chronic rat MITC exposure study conducted by [redacted] (2015a), which clearly demonstrated a threshold dose-level at 20 ppm for MITC, below which nasal tumors did not occur. Additionally, the first nasal tumors in the 20-ppm dose group were squamous cell carcinomas observed at weeks 39 in males.

The overall findings of MITC-induced nasal toxicity in multiple studies are consistent with our current understanding of cancer biology, mediated via chronic cytotoxicity, in that this process occurs over longer periods of time with continual exposure to the carcinogen. This is the case with the currently proposed

MOA, where a sequence of non-mutagenic key events, starting with cytotoxicity, leads to chronic tissue damage followed by reparative cell proliferation, hyperplasia, squamous metaplasia, and ultimately tumor formation.

Furthermore, recovery studies are informative tests for the strength of this association between exposure to MITC and the incidence of nasal tumors in rats, as they can demonstrate an absence or reduction of downstream key events when the exposure or an upstream key event is either blocked or diminished. In the acute rat MITC exposure study by (2011), partial resolution of cytotoxicity and acute inflammation was observed in an evaluation two weeks following the single, 6-hour exposure as shown in Table 3. The recovery groups from the (2020) study demonstrate that the exposure-related increase in nasal epithelial cell proliferation (measured as increased DNA synthesis) in the 5-ppm and 20-ppm groups was not persistent 28 days after the end of the last exposure. Further, the nasal lesions observed in the 5-ppm group (acute inflammation and epithelial cell hyperplasia) were no longer present following the 28-day post-exposure, suggesting a full recovery (2020). This was not the case with the 20-ppm group, in which nasal epithelial hyperplasia was still present in some of the 20-ppm exposed animals 28 days post-exposure, though of minimal to mild severity compared to mild to marked severity following 4 weeks of exposure suggesting that a longer recovery would lead to full reversibility.

Specificity addresses whether an exposure gives rise to a single outcome. For a chemical exposure, such as to MITC, this is much more challenging to prove than, for example, a viral exposure which may be caused by a single viral pathogen. While the 2-year rat MITC exposure study demonstrated an increase in nasal tumor incidence in Sprague Dawley rats (2015a), no increase in tumor incidence was observed in a similar study using CD-1 mice (2015b), even though several of the key events occurring in the rat were also described in the mouse. There are several physiological differences between the mouse and rat in terms of their ability to alter their respiratory rates, whereas the mouse is known to decrease its

respiratory rate when exposed to irritant chemicals (Larsen et al., 2016), differences in nasal dosimetry and, in combination with generally less severe nasal toxicity seen in mice, probably explains their resistance to developing nasal tumors. In addition, the mouse study was only for 18 months, not 24 months like the rat study, although the first carcinoma was detected in the rat study after 39 weeks. Further, there was no evidence of carcinogenicity either in mice or rats when MITC was administered via drinking water for 2 years (NRA, 1997), suggesting that the carcinogenicity of MITC may also be route-specific (inhalation versus oral). The lack of tumors systemically in the inhalation study also indicates that the tumorigenic response is localized to the site of exposure to the irritant, and it only occurs at the site of exposure. The cytotoxicity response was attenuated the further into the respiratory tract, where exposure to the irritant chemical will be decreased and ultimately below the threshold level required for irritation.

Biological Plausibility and Coherence

For the proposed MOA to be biologically plausible, it needs to be consistent with our current understanding of the biology of cancer. Non-genotoxic carcinogens act through mechanisms other than inducing DNA mutations as an initial key event. These non-genotoxic carcinogens act by increasing cell proliferation, either by direct mitogenesis or by cytotoxicity and regenerative cell proliferation. Direct mitogenesis has been demonstrated in animal models through activation of a variety of different receptors, including CAR (Elcombe et al., 2014; Yamada et al., 2021), AhR (Budinsky et al., 2014), PPARα (Corton et al., 2014; 2018), and estrogen (Preston-Martin et al., 1990). In humans, the only direct chemical mitogen that has been demonstrated to be carcinogenic is estrogen, which enhances the risk of cancer in breast and endometrium, as well as to a much lesser extent in the liver, and also in the ovary (Russo and Russo, 2006; Persson, 2000; Parazzini et al., 1991; SGO et al., 2014; Rooks et al., 1979; IARC, 1999).

Cytotoxicity and consequent regeneration (proliferation) are a common MOA for a variety of agents. Usually, the specific molecular initiating event is not known, but that is the actual molecular mechanism rather than the MOA. Cytotoxicity with regenerative proliferation has been identified as a common MOA for chemicals, especially in animal studies. Examples include chloroform related to liver and kidney tumors in rats and mice (Andersen et al., 2000), captan and folpet related to rat duodenal tumors (Cohen et al., 2010; Bhat et al., 2020), pulegone (da Rocha et al., 2012) and diuron (da Rocha et al., 2014) related to urinary bladder cancer, and in numerous other examples. In humans, cytotoxicity is a common event, with the most common example being hepatitis B and hepatitis C viruses related to chronic active hepatitis, cirrhosis, and ultimately the development of hepatocellular carcinoma (Knudsen et al., 2014).

For these non-genotoxic carcinogens, whether direct mitogenesis or cytotoxicity with regenerative increased cell proliferation occurs, there is a threshold in the dose response for the key events of the MOA.

This is to be expected since the events that lead to the ultimate development of tumors are non-neoplastic events. A risk assessment that establishes a dose that protects against the non-neoplastic lesions will also be protective for neoplastic development. These non-neoplastic risk assessments routinely involve the setting of a margin of exposure utilizing uncertainty factors, usually 100. The same approach can be used for the non-genotoxic carcinogens (Cohen et al., 2019).

In the reply from EPA regarding this MOA for MITC, they refer to an evaluation by suggesting that cytotoxicity and regenerative proliferation may not be the MOA for these nasal tumors. However, this ignores an enormous body of literature that indicates cytotoxicity and regenerative proliferation in a variety of tissues with a variety of agents can be the non-genotoxic MOA for cancer development. cited the example of glutaraldehyde as an indication that this MOA was not appropriate for MITC. He indicated that glutaraldehyde is highly cytotoxic when administered by inhalation but does not result in nasal cavity tumors. This is a superficial evaluation of glutaraldehyde as well as MITC, which requires further analysis. To begin with, while glutaraldehyde is highly cytotoxic, it is also genotoxic (Zeiger et al., 2005). It is not reasonable to claim that just because glutaraldehyde is genotoxic and not carcinogenic that genotoxicity cannot be a MOA for other chemicals. Likewise, it is also inappropriate to claim that because glutaraldehyde is highly cytotoxic and is not carcinogenic that the cytotoxicity cannot be a MOA for other chemicals. Furthermore, there is specific information available that is helpful in understanding the distinctions between glutaraldehyde (van Birgelen et al., 2000) and MITC, and for that matter, formaldehyde, which is also cytotoxic and genotoxic in the rat nasal cavity like glutaraldehyde but is carcinogenic (Andersen et al., 2019).

Additionally, glutaraldehyde in the 2-year bioassay by inhalation decreased survival in the treated animals, which certainly reduced the chances for detecting a carcinogenic effect. More importantly, however, the specific area of the nose that is affected by glutaraldehyde needs to be considered. Since glutaraldehyde is

extremely cytotoxic, even more so than MITC, it produces cytotoxicity essentially at the initial contact point of the nose following inhalation, rather than more distally in the nasal cavity. The most anterior part of the nose, where glutaraldehyde has its effect, is lined by normal squamous epithelium, not respiratory columnar epithelium. Glutaraldehyde by inhalation produces a marked inflammatory reaction and proliferation in this epithelium (Van Birgelen et al., 2000) with a negligible effect on the more distal respiratory epithelium since the glutaraldehyde has already reacted with the more anteriorly located squamous epithelium.

In contrast, while MITC is cytotoxic, it has little reaction with the initial contact in the squamous epithelium but reacts with the more distal respiratory columnar epithelium instead. With MITC, this very quickly results in squamous metaplasia as part of the regenerative and reparative process (see above). As mentioned above, although squamous metaplasia histologically resembles normal squamous epithelium, biologically they are distinctly different. Squamous metaplasia arising in glandular epithelium tissue, such as the respiratory epithelium of the nasal cavity, is where cancer typically arises instead of in the normal squamous epithelium, which appears to be much more resistant to the carcinogenicity of agents. This is similar to what is seen with uterine cervical cancer in women (Malpica, 2014; Trimble and Katzenellenbogen, 2014). The ectocervix is a normal squamous epithelium, and the endocervix is a glandular mucinous epithelium, somewhat similar to the respiratory epithelium in the nasal cavity. The initial reaction to HPV is transformation at the zone between endocervix and ectocervix leading to an inflammatory reaction and squamous metaplasia. The squamous cell carcinomas that develop in the cervix arise in this metaplastic zone, not in the normal squamous epithelium. The ectocervix, where the normal squamous cell epithelium is located, does not give rise to squamous tumors of the cervix in response to HPV infection. This is likely analogous to what occurs with MITC in the nasal cavity in rats. The tumors that arise with MITC inhalation are predominantly squamous cell carcinomas, with a few anaplastic carcinomas. Anaplastic carcinoma means that the tumors do not show a specific differentiation but are nevertheless arising from the

metaplastic squamous epithelium and can be thought of as a high-grade, fully differentiated squamous cell carcinoma.

Taken together, [redacted] implication that glutaraldehyde being cytotoxic but not carcinogenic forms a basis for concluding that MITC does not act by a cytotoxicity/regenerative proliferation MOA is unfounded. [redacted] evaluation does not take into account the specific location of the inflammatory regenerative proliferation that occurs with glutaraldehyde in the nasal cavity, nor does it recognize that glutaraldehyde is genotoxic. It would be equally inappropriate to conclude that genotoxicity cannot be a MOA for carcinogens simply because glutaraldehyde does not produce an increased incidence of cancer in the 2-year inhalation bioassay.

Increased cell proliferation is a well-known and established event that can lead to increased cancer risk. This was first stated by Armitage and Doll in 1954 (Armitage and Doll, 1954) in their seminal work relating the increased incidence of various cancers rising exponentially with age based on epidemiology studies. However, there are flaws with this model, especially the fact that there are a number of tumors that do not follow this age-related incidence, such as childhood tumors, Hodgkin's disease, and breast cancer. Other publications such as Moolgavkar and Knudson (1981), based on epidemiology studies, and Greenfield et al. (1984), based on animal studies, have developed more refined models that take into account changes in cell proliferation as well as DNA reactivity over time in order to consider these age-specific changes. It is well accepted that every time DNA replicates, mistakes occur spontaneously and very likely secondary to the numerous endogenous DNA adducts that are present in all cells (Chen and Ellwein, 1990; 1991; Swenberg et al., 2011; Cohen and Arnold, 2011). Over time, the multiple genetic mistakes that are necessary for cancer to develop will occur in a single cell (cancer is a clonal disease). Thus, any agent that increases the number of DNA replications (e.g., through cell proliferation) will increase the risk of cancer (Cohen and Ellwein 1990; 1991). The relationship of increased cell proliferation to cancer has been strongly

supported by subsequent research, most recently with studies in humans by Tomasetti and Vogelstein and their colleagues (Tomasetti and Vogelstein, 2015; Tomasetti et al., 2017), indicating that spontaneous errors occurring during normal DNA replication are more likely contributing to the cause of cancer in humans than are DNA reactive (mutagenic) agents. Overall, the MOA of cytotoxicity with consequent regenerative cell proliferation is not only biologically plausible, but strongly supported, by the evidence for MITC-induced nasal cavity tumors.

Possible Alternative MOAs

As discussed above, chemicals can potentially increase cancer risk by either reacting directly with DNA (DNA-reactive) or by increasing cell proliferation. Increased cell proliferation can occur either by direct mitogenesis or secondary to cytotoxicity and regenerative proliferation.

Only four possible MOAs have been documented for human carcinogenesis: 1) DNA reactivity; 2) immunosuppression; 3) increased estrogenic activity; and 4) cytotoxicity with regenerative proliferation (Cohen et al., 2019).

MITC is not DNA-reactive or genotoxic. A number of standard genotoxicity studies have been performed, which have been uniformly negative, including the bacterial reverse mutation assay, bacterial DNA damage and repair, *in vitro* UDS in rat hepatocytes, *in vitro* sister chromatid exchange in V79 fibroblasts, *in vitro* chromosomal aberration assay, and *in vivo* bone marrow micronucleus assay (see Appendix 1; Brusick, 2016). Table 9 lists studies reviewed by various regulatory authorities as reported in Brusick (2016). MITC was consistently negative in all these studies that used internationally accepted study protocols and multiple critical endpoints, except for an equivocal response, likely resulting from excessive cytotoxicity, in an *in vitro* chromosomal aberration assay.

Kassie et al. (2016) reported positive findings for several *in vitro* and *in vivo* genotoxicity endpoints (see Table 10). Based on a critical analysis of these studies, Brusick (2016) concluded that these studies suffered from technical and interpretation deficiencies and as such do not have any impact on the conclusion reached above regarding the non-genotoxicity of MITC.

Based on a weight-of-evidence analysis (Table 11), Brusick (2016) concluded that MITC is a non-genotoxic substance, a conclusion consistent with that of the EPA's (EPA, 2018).

Table 9: Summary of Genetic Toxicology Test Results for MITC Reviewed by Regulatory Authorities

Study	Author(s)	Report Date	Source	GLP	Genetic Endpoint	Reported Response	Appropriate MTD	Study Protocol
Ames	Glass *et al.*	1976	ANRA	No	Point Mutation	Negative	Yes	Inadequate (1 plate/dose)
Ames	Lang &Redmann	1978	ANRA	No	Point Mutation	Negative	Yes	Adequate
Ames	Shirasu *et al.*	1978	ANRA	No	Point Mutation	Negative	Possibly	Inadequate (used disk method)
Ames	Moriya *et al.*	1983	ANRA	No	Point Mutation	Negative	Yes	Adequate (same data likely published in a paper by Shirasu in 1982)
Ames	Glebke and Engelhardt	1986	ANRA	No	Point Mutation	Negative	Yes	Adequate
V79 cell Hprt Assay	Miltenburger	1985	ANRA	Yes	Point Mutation	Negative	Yes	Sufficient
In vitro rat hepatocyte UDS	Cifone	1985	ANRA	Yes	Induction of DNA repair	Negative	Yes	Sufficient
Clastogenicity in V79 cells *in vitro*	Miltenburger	1985	ANRA	Yes	Aberration analysis	Equivocal	Probably not	Inadequate (Preliminary cytotoxicity test suggests MTD exceeded for –S9 trials and likely for +S9 trials)
Clastogenicity in human lymphocyte cultures	Heideman	1988	CAEPA	?	Aberration analysis	Negative	Yes	Adequate
Clastogenicity in human lymphocyte cultures	Glebke and Engelhardt	1987	ANRA	No	Aberration analysis	Negative	Yes	Adequate
Sister Chromatid Exchange in V79 cells *in vitro*	Heidemann	1988	CAEPA	?	SCE	Negative	Yes	Adequate
REC Assay	Shirasu	1978	ANRA	No	Differential survival in *B. subtilis* normal and repair deficient bacteria	Negative	Yes	Adequate
Rec Assay	Jagannath	1989	ANRA	Yes	Differential survival in *B. subtilis* normal and repair deficient bacteria	Negative	Yes	Sufficient
In vivo micronucleus test in mice	Allen *et al.*	1985	ANRA	Yes	Induction of micronuclei in mouse bone marrow cells	Negative	Yes (single oral dose of 110 mg/kg)	Sufficient design with 3 harvest times of 24, 48 and 72 hours.
In vivo micronucleus test in mice	Weinberg	2011	USEPA-1	Yes	Induction of micronuclei in mouse bone marrow cells	Negative	Yes (inhalation exposure)	Sufficient

Footnotes:
1. ANRA = Australian National Registration Authority, NRA Special Review Series 97. 2, 1997
2. CAEPA = California EPA Evaluation of MITC, Part C: Human Health Assessment, 2002
3. USEPA -1= United States EPA, Memo dated 2012; USEPA-2 = United States EPA, Memo dated 2004
4. Inadequate = study design not sufficient to fully support the reported results; Adequate = study design was technically sound and state of the art at the time the study was conducted, but may not meet current standards and may not comply with GLP regulations; Sufficient = study likely meets current guideline and GLP requirements.

Table 10. Review of Genetic Toxicology Tests Reported by Kassie et al., 2001.

Study	GLP	Genetic Endpoint	Reported Response	Appropriate MTD	Study Protocol	Assessment
Ames Test	No	Point Mutation	Indications of positive effects in agar overlay (–S9) method but not using a pre-incubation method	Yes	Data were presented graphically not allowing for adequate assessment. The response did not achieve current criteria for classification as positive effect in either strain.	Study is negative for mutation
Micronucleus test *in vitro*	No	MN in HepG2 cells	Positive	50% cell viability at 4 µg/ml is acceptable	Adequate	Positive study
Comet Assay *in vitro*	No	Induction of single/double strand DNA break in HepG2 cells	Positive	The concentration of 5.4 µg/ml is likely too toxic based on toxicity curve provided for MN test.	There was no toxicity data provided. Using the data from the MN test, a concentration of 5.4 µg/ml would already be at less than 70% survival for a 1-hour exposure. The data shown for this study did not give lower confidence limits nor statistical assessment for the low dose of 3.65 µg/ml. The data from this study cannot be reliably interpreted.	Limited value for assessing genotoxic potential
Host-Mediated Assay	No	Comparative survival between repair deficient strain, 343/753 (uvrB/recA) and the repair proficient counterpart, 343/765 (uvr+/rec+). Following i.v. injection of strains into mice	Positive response reported for bacteria isolated from liver tissue but negative in lung, kidney, colon, stomach.	A dose of 270 mg/kg killed all test animals within 2 hours. The toxicity of the lower dose level was not given in the paper.	This study does not provide data that is can be reliably interpreted. Based on 100% lethality of mice at 270 mg/kg, that 90 mg/kg would give rise to endogenous toxic effects that would interfere with responses of seen in bacteria. In addition, the variability across survival in the control animals overlapped the range among the treated animals. Data cannot be reliably interpreted	Value for assessing genotoxic potential is limited to none
Bacteria DNA repair test	No	Comparative survival between repair deficient strain, 343/753 (uvrB/recA) and the repair proficient counterpart, 343/765 (uvr+/rec+).	Positive	Yes	Adequate	Value for assessing genotoxic potential is limited to none
Tbars Test	No	Assessing oxidative stress in HepG2 cells	Positive	NA	Adequate	Data indicate that MITC will induce oxidative intermediates *in vitro*

Table 11. Weight-of-Evidence Summary of Responses

Test Method	Genetic Endpoint	Relevance to Genotoxic Hazard/Risk	Number of Studies (Tables 1 and 2)	Response(s) = Positive/Negative (invalid)	Comment
Ames Tests	Gene Mutation	High	6	0/6	Based on detailed critique
Hprt Assay in V79 Cells	Gene Mutation	High	1	0/1	
In Vitro Clastogenicity in Cell Lines (V79/HepG2)	Chromosome aberrations and/or micronuclei formation	Moderate-High	2	1/1	Based on detailed critique
In Vitro Clastogenicity in Human Lymphocytes	Chromosome aberrations	Moderate-High	2	0/2	
In Vivo Clastogenicity in Mice	Micronuclei formation	High	2	0/2	
UDS in Rat Hepatocytes In Vitro	Indirect measure of DNA repair	Moderate-low	1	0/1	
In Vitro SCEs in V79 Cells	Sister Chromatid Exchange	Low	1	0/1	
In Vitro Comet Assay in HepG2 Cells	Single/double strand breaks in DNA	Low	1	0/(1)	Technical issues limit interpretation of this test
Differential Survival in Repair Sufficient and Deficient Bacteria In Vitro	Comparison of toxic response curves in repair deficient and normal bacteria	Low	3	1/2	
Host-mediated Assay	Comparison of toxic response curves in repair deficient and normal bacteria isolated from tissue	None	1	0/(1)	Technical issues limit interpretation of this test
Totals			**20**	**2/18 (90% negative or invalid responses)**	

Immunosuppression increases the risk of certain cancers, regardless of the process that leads to the immunosuppression (e.g., due to inherited disorders, pharmaceutical treatment for a variety of disorders such as transplantation, cancer chemotherapy, treatment of autoimmune diseases, or AIDS) (Penn, 1988; Cohen et al., 1991; Busnack et al., 2006; Grulich et al., 2007; Engels et al., 2011; Simard et al., 2012; Krynitz et al., 2019). However, only a limited number of cancers are increased in immunosuppressed individuals, and these are mostly related to viral disorders. Some examples include various squamous epithelial cancers related to HPV infection, lymphomas and a variety of less common tumors related to EBV, Kaposi sarcoma related to KSV (also called HHV8), and liver cancer related to hepatitis B or C viruses. There also appears to be an increased risk of malignant melanoma likely related to the high mutagenicity and antigenicity activities in these tumors. Also, in kidney transplant recipients there is an increased incidence of kidney tumors, although this is most likely related to the chronic kidney disease rather than immunosuppression. Nevertheless, nasal cavity tumors are not among the tumors that are at increased risk in immunosuppressed individuals. It should be noted that immunosuppressive agents are poorly detected in 2-year animal bioassays (Bagalski et al., 2010). For MITC, there is no evidence of an effect on immune or hematologic tissues, such as thymus, bone marrow, spleen, gastrointestinal tract, or tonsils in the sub-chronic and chronic studies reviewed in here. Taken together, immunosuppression can be excluded as a MOA for MITC-induced nasal cavity tumors.

Estrogenic activity has not been demonstrated for MITC, and in the animal bioassays there has been no effect on estrogen-dependent tissues such as the mammary gland, ovaries, and uterus ([redacted] 2015a; 2015b). It is therefore highly unlikely that MITC is producing nasal cavity tumors by an estrogenic effect. Furthermore, increased estrogen is only associated with a limited number of tumors in humans, including breast, endometrium, ovary, and to a lesser extent liver (Russo and Russo, 2006; Persson, 2000; Parazzini et al., 1991; SGO et al., 2014; Rooks et al., 1979; IARC, 1999). Thus, estrogenic activity can be excluded as a MOA for MITC-induced nasal cavity tumors.

Considering each of the potential MOAs that have been documented for human cancers, none other than the cytotoxicity with regenerative proliferation MOA is supported by the available data for MITC exposure through the inhalation route. Furthermore, the evidence is strong, coherent, consistent, and is certainly biologically plausible.

As part of a cytotoxicity response, there may be an effect on oxidative stress as well as on glutathione levels (Burke et al., 2007; Jaeschke et al., 2021). These effects are likely to occur in response to MITC exposure, but are a consequence of MITC cytotoxicity and not a MOA, *per se*, for the nasal cavity tumors. Increased levels of oxidative stress are routinely seen with chemicals that produce cytotoxicity (Burke et al., 2007; Jaeschke et al., 2021) even if they are not related to carcinogenicity, such as in the case of acetaminophen, which is generally regarded as non-carcinogenic (Jaeschke et al., 2021; Murray et al., 2020). The relationship of oxidative stress and cancer is complex and by itself does not represent a cancer MOA. In fact, antioxidants can actually enhance the risk of cancer. For example, studies evaluating antioxidants in double blind clinical studies have lacked preventive effects or actually enhanced the risk of cancer (Alpha Tocopherol, Beta Carotene Prevention Study Group, 1994; Albanes et al., 1996; Omenn et al., 1996; Albanes, 2009). In addition, some antioxidants have been shown to be carcinogenic in animal models, such as butylated hydroxyanisole (BHA) and related phenolic antioxidants (Ito and Hirose, 1989). Given the strong evidence for cytotoxicity and regenerative proliferation as the MOA, and no evidence for alternative MOAs, the support for cytotoxic regenerative proliferation as the MOA for these tumors is strong.

Uncertainties, Inconsistencies, and Data Gaps

In general, the toxicological database for MITC addresses the critical aspects of the proposed MOA for nasal tumors in rats, as well as evaluation of alternative MOAs. The data are consistent and demonstrate dose-response and temporal concordance across key events for the development of nasal tumors in the rat (see Table 8).

An uncertainty of the proposed MOA is the absence of tumors in the mouse bioassay, given that MITC is expected to cause, and the data supports, cytotoxicity and irritants in the nasal passages of mice following inhalation exposures. Currently, the contribution from the dosimetric differences between the rat and the mouse nasal passages, the duration of exposure, the generally lower severity in incidence of the findings in the mouse, as well as the known physiological differences in the respiratory parameters between these two species and the observed differences in the tumor response is unknown. The mouse bioassay was only for 18 months compared to the 24 months in the rat bioassay, although the first tumor in the rat was detected at 39 weeks.

The specific molecular events leading to the cytotoxicity are not known, but this is a deficiency that is usually found with cytotoxicity. As indicated above, oxidative stress as well as glutathione depletion are aspects of cytotoxicity and not necessarily the cause; as such, the lack of data on these endpoints in the nasal passages of MITC-exposed rats is not considered to be a data gap in the assessment of the MOA. Furthermore, these changes may occur with any form of cytotoxicity, even if it does not lead to tumor formation.

Human Applicability of the Proposed MOA

Is the Weight of Evidence Sufficient to Establish a MOA in the Rat?

The weight of evidence is sufficient to establish a MOA in the rat. While the complete mechanistic details of the MOA may not all be available, data from the available inhalation studies with MITC as well as other irritant compounds reinforce confidence in the cytotoxicity-based MOA for nasal tumors in rats.

Can Human Relevance of the MOA be Reasonably Excluded on the Basis of Fundamental Differences in Key Events Between Rats and Humans?

The answer is "no". In general, the proposed MOA for MITC-induced rat nasal tumors is potentially applicable to humans as well, since MITC is known to be an irritant for humans. Accordingly, tumorigenicity is expected in humans if they are chronically exposed to high enough concentrations of MITC that can induce excessive cytotoxicity leading to continuous reparative increased cell proliferation.

Can Human Relevance of the MOA be Reasonably Excluded on the Basis of Quantitative Differences Either Kinetic or Dynamic Factors Between Rats and Humans?

The answer is "yes". Chronic exposures to high levels of MITC leading to excessive cytotoxicity in the nasal passages are not expected to occur in real life situations in humans. For example, the estimated health protective concentration per irritant effects of acute (4-8 hours) MITC exposure is in the range of 0.2 to 0.8 ppm and is based on the effects (perceived magnitude of irritation, blinking, and tearing) observed in a human study by [redacted] (1996) ([redacted] 2010; EPA, 2008). Thus, the concentration required to induce tumors in the rat chronic toxicity is at least 25X higher than the health protective value for acute irritation in humans. Tumorigenic consequences are a product of prolonged exposure to high, and excessively irritating, concentrations of MITC which individuals would not be expected to tolerate for any

length of time. At exposure concentrations and durations that do not induce successive sensory irritancy, cytotoxicity and the consequent sustained increase reparative cell proliferation, MITC would not be expected to induce tumors in humans.

Conclusions

Inhalation of 20 ppm MITC (tumorigenic concentration) induced nasal cavity lesions in the rat consisting of acute/subacute inflammation, epithelial cell degeneration/necrosis, increased epithelial cell proliferation (increased DNA synthesis detected by nuclear incorporation of BrdU, hyperplasia, and regeneration), olfactory sensory neuron apoptosis, and olfactory sensory neuron loss resulting in atrophy/loss of olfactory epithelium and respiratory metaplasia. These results along with significant decrements in body weight (up to 34%), and body weight gain (up to 47%) in the two-year study, indicate that the 20-ppm concentration was toxic to the rats. However, EPA has determined that the tumors are treatment related and 20 ppm did not exceed the MTD.

Analysis of available toxicity data from the rat inhalation toxicity studies indicated that a biologically plausible MOA leading to nasal tumors at the 20 ppm exposure level involved the following the key events: 1) direct cytotoxicity of MITC to the nasal mucosa (degeneration, necrosis/apoptosis); 2) consequent increased cell proliferation to compensate for MITC-induced cytotoxicity; 3) on-site and persistence of squamous cell metaplasia of the respiratory mucosa as an adaptive response to the cytotoxic insult; and 4) development of tumors. This analysis provided confidence in the proposed MOA for MITC-induced rat nasal tumors and further informed that the tumorigenicity of MITC has a threshold. This conclusion is further supported by the recent EU review where the RMS concluded that the tumorigenicity is "clearly threshold-like" and "obviously triggered by the corrosive action of MITC in the nasal linings" (RMS Belgium, 2021).

Furthermore, MITC is not likely to pose a carcinogenic hazard or risk to humans under normal environmental or occupational exposures. Detailed analysis showed that there was a dose response and temporal response concordance between the key events and the ultimate toxicity (tumor formation), with

the strength of the response being strong. The findings between studies were consistent and specific. The MOA of cytotoxicity with consequent regenerative increased cell proliferation is a well-known MOA for chemicals, so it is biologically plausible and coherent with what is known in biology. Alternative MOAs have been excluded, including DNA reactivity, immunosuppression, and estrogenic activity. Given that the MOA involves sustained cytotoxic and regenerative response with squamous metaplasia in response to chronic irritation, which is not expected to occur in humans (because of the limited levels of exposure as discussed above), this MOA does not pose a cancer hazard or risk to humans.

References

Albanes, D., Heinonen, O. P., Taylor, P. R., Virtamo, J., Edwards, B. K., Rautlahti, M., Hartman, A. M., Palmgren, J., Freedman, L. S., Haapakoski, J., Barrett, M. J., Pietinen, P., Malila, N., Tala, E., Liippo, K., Salomaa, E. R., Tangrea, J. A., Teppo, L., Askin, F. B., Taskinen, E., Erozan, Y., Greenwald, P., Huttunen, J. K. Alpha-tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. J. Natl. Cancer Inst. 88(21):1560-1570, 1996. DOI: 10.1093/jnci/88.21.1560

Albanes, D. Vitamin supplements and cancer prevention: where do randomized controlled trials stand? J. Natl. Cancer Inst. 101(1):2-4, 2009. DOI: 10.1093/jnci/djn453

Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. N. Engl. J. Med. 330(15):1029-1035, 1994. DOI: 10.1056/nejm199404143301501

Andersen, M. E., Meek, M. E., Boorman, G. A., Brusick, D. J., Cohen, S. M., Dragan, Y. P., Frederick, C. B., Goodman, J. I., Hard, G. C., O'Flaherty, E. J., Robinson, D. E. Lessons learned in applying the U.S. EPA proposed cancer guidelines to specific compounds. Toxicol. Sci. 53(2):159-172, 2000. DOI: 10.1093/toxsci/53.2.159

Andersen, M. E., Gentry, P. R., Swenberg, J. A., Mundt, K. A., White, K. W., Thompson, C., Bus, J., Sherman, J. H., Greim, H., Bolt, H., Marsh, G. M., Checkoway, H., Coggon, D., Clewell 3rd, H. J. Considerations for refining the risk assessment process for formaldehyde: results from an interdisciplinary workshop. Regul. Toxicol. Pharmacol. 106:210-223, 2019. DOI: 10.1016/j.yrtph.2019.04.015

Armitage, P., Doll, R. The age distribution of cancer and a multi-stage theory of carcinogenesis. Br. J. Cancer 8(1):1-12, 1954. DOI: 10.1038/bjc.1954.1

Bhat, V. S., Cohen, S. M., Gordon, E. B., Wood, C. E., Cullen, J. M., Harris, M. A., Proctor, D. M., Thompson, C. M. An adverse outcome pathway for small intestine tumors in mice involving chronic cytotoxicity and regenerative hyperplasia: a case study with hexavalent chromium, captan, and folpet. Crit. Rev. Toxicol. 50(8):685-706, 2020. DOI: 10.1080/10408444.2020.1823934.

Brusick, D. 2016. As assessment of the genotoxic properties of methyl isothiocyanate (MITC). A report prepared for the MITC Task Force.

Boobis, A. R., Cohen, S. M., Dellarco, V., McGregor, D., Meek, M. E. B., Vickers, C., Willcocks, D., Farland, W. IPCS framework for analyzing the relevance of a cancer mode of action for humans. Crit. Rev. Toxicol. 36(10):781-792, 2006. DOI: 10.1080/10408440600977677

Boobis, A. R., Doe, J. E., Heinrich-Hirsch, B., Meek, M. E. B., Munn, S., Ruchirawat, M., Schlatter, J., Seed, J., Vickers, C. IPCS framework for analyzing the relevance of a noncancer mode of action for humans. Crit. Rev. Toxicol. 38(2):87-96, 2008. DOI: 10.1080/10408440701749421

Budinsky, R. A., Schrenk, D., Simon, T., Van den Berg, M., Reichard, J. F., Silkworth, J. B., Aylward, L. L., Brix, A., Gasiewicz, T., Kaminski, N., Perdew, G., Starr, T. B., Walker, N. J., Rowlands, J. C. Mode of

action and dose-response framework analysis for receptor-mediated toxicity: the aryl hydrocarbon receptor as a case study. Crit. Rev. Toxicol. 44(1):83-119, 2014. DOI: 10.3109/10408444.2013.835787

Bugelski, P. J., Volk, A., Waalker, M. R., Krayer, J. H., Martin, P., Descotes, J. Critical review of preclinical approaches to evaluate the potential of immunosuppressive drugs to influence human neoplasia. Int. J. Toxicol. 29(5):435-466, 2010. DOI: 10.1177/1091581810374654

Burke, A. S., Redeker, K., Kurten, R. C., James, L. P., Hinson, J. A. Mechanisms of chloroform-induced hepatotoxicity: oxidative stress and mitochondrial permeability transition in freshly isolated mouse hepatocytes. J. Toxicol. Environ. Health A. 70(22):1936-1945, 2007. DOI: 10.1080/15287390701551399

Busnach, G., Piselli, P., Arbustini, E., Baccarani, U., Burra, P., Carrieri, M. P., Citterio, F., De Juli, E., Bellelli, S., Pradier, C., Rezza, G., Serraino, D, Immunosuppression and Cancer Study Group. Immunosuppression and cancer: a comparison of risks in recipients of organ transplants and in HIV-positive individuals. Transplant Proc. 38(10):3533-3535, 2006. DOI: 10.1016/j.transproceed.2006.10.144

Cohen, S. M., Arnold, L. L. Chemical carcinogenesis. Toxicol. Sci. 120 Suppl 1:S76-92, 2011. DOI: 10.1093/toxsci/kfq365

Cohen, S. M., Ellwein, L. B. Cell proliferation in carcinogenesis. Science 249(4972):1007-1011, 1990. DOI: 10.1126/science.2204108

Cohen, S. M., Ellwein, L. B. Genetic errors, cell proliferation, and carcinogenesis. Cancer Res. 51(24):6493-6505, 1991. PMID: 1742722

Cohen, S. M., Purtilo, D. T., Ellwein, L. B. Ideas in pathology. Pivotal role of increased proliferation in human carcinogenesis. Mod. Pathol. 4(3):371-382, 1991. PMID: 2068065

Cohen, S. M., Klaunig, J. Meek, M. E., Hill, R. N., Pastoor, T., Lehman-McKeeman, L., Bucher, J., Longfellow, D. G., Seed, J., Dellarco, V., Fenner-Crisp, P., Patton, D. Evaluating the human relevance of chemically induced animal tumors. Toxicol. Sci. 78(2):181-186, 2004. DOI: 10.1093/toxsci/kfh073

Cohen, S. M., Gordon, E. B., Singh, P., Arce, G. T., Nyska, A. Carcinogenic mode of action of folpet in mice and evaluation of its relevance to humans. Crit. Rev. Toxicol. 40(6):531-545, 2010. DOI: 10.3109/10408441003742903

Cohen, S. M., Boobis, A. R., Dellarco, V. L., Doe, J. E., Fenner-Crisp, P. A., Moretto, A., Pastoor, T. P., Schoeny, R. S., Seed, J. G., Wolf, D. C. Chemical carcinogenicity revisited 3: risk assessment of carcinogenic potential based on the current state of knowledge of carcinogenesis in humans. Regul. Toxicol. Pharmacol. 103:100-105, 2019. DOI: 10.1016/j.yrtph.2019.01.017

Corton, J. C., Cunningham, M. L., Hummer, B. T., Lau, C., Meek, B., Peters, J. M., Popp, J. A., Rhomberg, L., Seed, J., Klaunig, J. E. Mode of action framework analysis for receptor-mediated toxicity: the peroxisome proliferator-activated receptor alpha (PPARα) as a case study. Crit. Rev. Toxicol. 44(1):1-49, 2014. DOI: 10.3109/10408444.2013.835784

Corton, J. C., Peters, J. M., Klaunig, J. E. The PPARα-dependent rodent liver tumor response is not relevant to humans: addressing misconceptions. Arch. Toxicol. 92(1):83-119, 2018. DOI: 10.1007/s00204-017-2094-7

da Rocha, M., Dodmane, P. R., Arnold, L. L., Pennington, K. L., Anwar, M. M., Adams, B. R., Taylor, S. V., Wermes, C., Adams, T. B., Cohen, S. M. Mode of action of pulegone on the urinary bladder of F344 rats. Toxicol. Sci. 128(1):1-8, 2012. DOI: 10.1093/toxsci/kfs135

da Rocha, M. S., Arnold, L. L., De Oliveira, M. L. C. S., Catalano, S. M. I., Cardoso, A. P. F., Pontes, M. G. N., Ferrucio, B., Dodmane, P. R., Cohen, S. M., De Camargo, J. L. Diuron-induced rat urinary bladder carcinogenesis: mode of action and human relevance evaluations using the International Programme on Chemical Safety framework. Crit. Rev. Toxicol. 44(5):393-406, 2014. DOI: 10.3109/10408444.2013.877870

Elcombe, C. R., Peffer, R. C., Wolf, D. C., Bailey, J., Bars, R., Bell, D., Cattley, R. C., Ferguson, S. S., Geter, D., Goetz, A., Goodman, J. I., Hester, S., Jacobs, A., Omiecinski, C. J., Schoeny, R., Xie, W., Lake, B. G. Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: a case study with phenobarbital as a model constitutive androstane receptor (CAR) activator. Crit. Rev. Toxicol. 44(1):64-82, 2014. DOI: 10.3109/10408444.2013.835786

Engels, E. A., Pfeiffer, R. M., Fraumeni Jr., J. F., Kasiske, B. L., Israni, A. K., Snyder, J. J., Wolfe, R. A., Goodrich, N. P., Bayakly, A. R., Clarke, C. A., Copeland, G., Finch, J. L., Fleissner, M. L., Goodman, M. T., Kahn, A., Koch, L., Lynch, C. F., Madeleine, M. M., Pawlish, K., Rao, C., Williams, M. A., Castenson, D., Curry, M., Parsons, R., Fant, G., Lin, M. Spectrum of cancer risk among US solid organ transplant recipients. JAMA 306(17):1891-1901, 2011. DOI: 10.1001/jama.2011.1592.

EPA (2018). Methyl Isothiocyanate (MITC): Cancer Assessment Review Committee's Third Report, Decision No. 515778, PC Bar Code 068103, Office of the Chemical Safety and Pollution Prevention, Washington, DC.

Greenfield, R. E., Ellwein, L. B., Cohen, S. M. A general probabilistic model of carcinogenesis: analysis of experimental urinary bladder cancer. Carcinogenesis 5(4):437-445, 1984. DOI: 10.1093/carcin/5.4.437

Grulich, A. E., van Leeuwen, M. T., Falster, M. O., Vajdic, C. M. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. Lancet 370(9581):59-67, 2007. DOI: 10.1016/s0140-6736(07)61050-2

Harkema, J. R., Carey, S. A., Wagner, J. G. The nose revisited: a brief review of the comparative structure, function, and toxicologic pathology of the nasal epithelium. Toxicol. Pathol. 34(3):252-269, 2006. DOI: 10.1080/01926230600713475

Hill, A. B. The environment and disease: association or causation? Proc. R. Soc. Med. 58(5):295-300, 1965. PMID: 14283879

IARC (1999). IARC monographs on the evaluation of carcinogenic risk to humans. Volume 72. Hormonal Contraception and post-menopausal hormone therapy. Lyon, France

Ito, N., Hirose, M. Antioxidants—carcinogenic and chemopreventive properties. Adv. Cancer Res. 53:247-302, 1989. DOI: 10.1016/s0065-230x(08)60283-3

Jaeschke, H., Murray, F. J., Monnot, A. D., Jacobson-Kram, D., Cohen, S. M., Hardisty, J. F., Atillosoy, E., Hermanowiski-Vosatka, A., Kuffner, E., Wikoff, D., Chappell, G. A., Bandara, S. B., Deore, M.,

Pitchaiyan, S. K., Eichenbaum, G. Assessment of the biochemical pathways for acetaminophen toxicity: implications for its carcinogenic hazard potential. Regul. Toxicol. Pharmacol. 120:104859, 2021. DOI: 10.1016/j.yrtph.2020.104859

Johnson, N. F., Hotchkiss, J. A., Harkema, J. R., Henderson, R. F. Proliferative responses of rat nasal epithelia to ozone. Toxicol. Appl. Pharmacol. 103(1):143-155, 1990. DOI: 10.1016/0041-008x(90)90270-5.

Kassie F, Laky B, Nobis E, Kundi M, Knasmüller S. Genotoxic effects of methyl isothiocyanate. Mutat Res. 2001 Jan 25;490(1):1-9. doi: 10.1016/s1383-5718(00)00140-6. Erratum in: Mutat Res 2001 May 31;492(1-2):111-3. PMID: 11152966.

Knudsen, E. S., Gopal, P., Singal, A. G. The changing landscape of hepatocellular carcinoma: etiology, genetics, and therapy. Am. J. Pathol. 184(3):574-583, 2014. DOI: 10.1016/j.ajpath.2013.10.028

Krynitz, B., Edgren, G., Lindelöf, B., Baecklund, E., Brattström, C., Wilczek, H., Smedby, K. E. Risk of skin cancer and other malignancies in kidney, liver, heart and lung transplant recipients 1970 to 2008 -- a Swedith population-based study. Int. J. Cancer 132(6):1429-1438, 2013. DOI: 10.1002/ijc.27765

Malpica, A. Cervical benign and non-neoplastic conditions. IN: Mutter, G. L., Prat, J. Pathology of the Female Reproductive Tract. Churchill Livingstone, Elsevier, chapter 8, pp. 160-187, 2014

Meek, M. E. B., Bucher, J. R., Cohen, S. M., Dellarco, V., Hill, R. N., Lehman-McKeeman, L. D., Longfellow, D. G., Pastoor, T., Seed, J., Patton, D. E. A framework for human relevance analysis of information on the carcinogenic modes of action. Crit. Rev. Toxicol. 33(6):591-653, 2003. DOI: 10.1080/713608373

Moolgavkar, S. H., Knudson Jr., A. G. Mutation and cancer: a model for human carcinogenesis. J. Natl. Cancer Inst. 66(6):1037-1052, 1981. DOI: 10.1093/jnci/66.6.1037

Murray, F. J., Monnot, A. D., Jacobson-Kram, D., Cohen, S. M., Hardisty, J. F., Bandara, S. B., Kovochich, M., Deore, M., Pitchaiyan, S. K., Gelotte, C. K., Lai, J. C. K., Atillosoy, E., Hermanowski-Vosatka, A., Kuffner, E., Unice, K. M., Yang, K., Gebremichael, Y., Howell, B. A., Eichenbaum, G. A critical review of the acetaminophen preclinical carcinogenicity and tumor promotion data and their implications for its carcinogenic hazard potential. Regul. Toxicol. Pharmacol. 118:104801, 2020. DOI: 10.1016/j.yrtph.2020.104801

Omenn, G. S., Goodman, G. E., Thornquist, M. D., Balmes, J., Cullen, M. R., Glass, A., Keogh, J. P., Meyskens, F. L., Valanis, B., Williams, J. H., Barnhart, S., Hammar, S. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N. Engl. J. Med. 334(18):1150-1155, 1996. DOI: 10.1056/nejm199605023341802

Parazzini, F., La Vecchia, C., Bocciolone, L., Francesci, S. The epidemiology of endometrial cancer. Gynecol. Oncol. 41(1):1-16, 1991. DOI: 10.1016/0090-8258(91)90246-2

Penn, I. Tumors of the immunocompromised patient. Annu. Rev. Med. 39:63-73, 1988. DOI: 10.1146/annurev.me.39.020188.000431

Persson, I. Estrogens in the causation of breast, endometrial and ovarian cancers -- evidence and hypotheses from epidemiological findings. J. Steroid Biochem. Mol. Biol. 74(5):357-364, 2000. DOI: 10.1016/s0960-0760(00)00113-8

Preston-Martin, S., Pike, M. C., Ross, R. K., Jones, P. A., Henderson, B. E. Increased cell division as a cause of human cancer. Cancer Res. 50(23):7415-7421, 1990. PMID: 2174724

Renne, R., Brix, A., Harkema, J., Herbert, R., Kittel, B., Lewis, D., March, T., Nagano, K., Pino, M., Rittinghausen, S., Rosenbruch, M., Tellier, P., Wohrmann, T. Proliferative and nonproliferative lesions of the rat and mouse respiratory tract. Toxicol. Pathol. 37(7 Suppl):5S-73S, 2009. DOI: 10.1177/0192623309353423

RMS Belgium. Draft Renewal Assessment Report prepared by RMS Belgium in the context of the application for renewal of approval of the active substance according to Reg. (EU) No 844/2012 (version dated 23-07-2021, unpublished)

Rooks, J. B., Ory, H. W., Ishak, K. G., Strauss, L. T., Greenspan, J. R., Hill, A. P., Tyler Jr., C. W. Epidemiology of hepatocellular adenoma. The role of oral contraceptive use. JAMA 242(7):644-648, 1979. PMID: 221698

Russo, J., Russo, I. H. The role of estrogen in the initiation of breast cancer. J. Steroid Biochem. Mol. Biol. 102(1-5):89-96, 2006. DOI: 10.1016/j.jsbmb.2006.09.004

SGO Clinical Practice Endometrial Cancer Working Group, Burke, W. M., Orr, J., Leitao, M, Salom, E., Gehrig, P., Olawaiye, A. B., Brewer, M., Boruta, D., Villella, J., Herzog, T., Shahin, F. A., Society of Gynecologic Oncology Clinical Practice Committee. Endometrial cancer: a review and current management strategies: part I. Gynecol. Oncol. 134(2):385-392, 2014. DOI: 10.1016/j.ygyno.2014.05.018

Seed, J., Carney, E. W., Corley, R. A., Crofton, K. M., DeSesso, J. M., Foster, P. M. D., Kavlock, R., Kimmel, G., Klaunig, J., Meek, M. E., Preston, R. J., Slikker Jr., W., Tabacova, S., Williams, G. M., Wiltse, J., Zoeller, R. T., Fenner-Crisp, P., Patton, D. E. Overview: using mode of action and life stage information to evaluate the human relevance of animal toxicity data. Crit. Rev. Toxicol. 35(8-9):664-672, 2005. DOI: 10.1080/10408440591007133

Simard, E. P., Shiels, M. S., Bhatia, K., Engels, E. A. Long-term cancer risk among people diagnosed with AIDS during childhood. Cancer Epidemiol. Biomarkers Prev. 21(1):148-154, 2012. DOI: 10.1158/1055-9965.epi-11-0823

Sonich-Mullin, C., Fielder, R., Baetcke, K., Dempsey, J., Fenner-Crisp, P., Grant, D., Hartley, M., Knaap, A., Kroese, D., Mangelsdorf, I., Meek, E., Rice, J. M., Younes, M., International Programme on Chemical Safety. Regul. Toxicol. Pharmacol. 34(2):146-152, 2001. DOI: 10.1006/rtph.2001.1493

Swenberg, J. A., Lu, K., Moeller, B. C., Gao, L., Upton, P. B., Nakamura, J., Starr, T. B. Endogenous versus exogenous DNA adduts: their role in carcinogenesis, epidemiology, and risk assessment. Toxicol. Sci. 120 Suppl 1:S130-145, 2011. DOI: 10.1093/toxsci/kfq371

Tomasetti, C., Vogelstein, B. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. Science 347(6217):78-81, 2015. DOI: 10.1126/science.1260825

Tomasetti, C., Li, L., Vogelstein, B. Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention. Science 355 (6331):1330-1334, 2017. DOI: 10.1126/science.aaf9011

Trimble, C. L., Katzenellenbogen, R., Biology of cervical squamous neoplasm. IN: Mutter, G. L., Prat, J., Pathology of the Female Reproductive Tract. Churchill Livingstone, Elsevier, chapter 9, pp. 188-199, 2014

van Birgelen, A. P., Chou, B. J., Renne, R. A., Grumbein, S. L., Roycroft, J. H., Hailey, J. R., Bucher, J. R. Effects of glutaraldehyde in a 2-year inhalation study in rats and mice. Toxicol. Sci. 55(1):195-205, 2000. DOI: 10.1093/toxsci/55.1.195

Wood, C. E., Hukkanen, R. R., Sura, R., Jacobson-Kram, D., Nolte, T., Odin, M., Cohen, S. M. Scientific and Regulatory Policy Committee (SRPC) review: interpretation and use of cell proliferation data in cancer risk assessment. Toxicol. Pathol. 43(6):760-775, 2015. DOI: 10.1177/0192623315576005

Yamada, T., Cohen, S. M., Lake, B. G. Critical evaluation of the human relevance of the mode of action for rodent liver tumor formation by activators of the constitutive androstane receptor (CAR). Crit. Rev. Toxicol. 51(5):373-394, 2021. DOI: 10.1080/10408444.2021.1939654

Zeiger, E., Gollapudi, B., Spencer, P. Genetic toxicity and carcinogenicity studies of glutaraldehyde -- a review. Mutat. Res. 589(2):136-151, 2005. DOI: 10.1016/j.mrrev.2005.01.001