

Selected Sections from the External Peer Review Draft
IRIS Toxicological Review for Acrylonitrile:
Highlighted text showing changes based on recent
EPA decisions regarding Ramazzini Institute studies

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4.2.1.2.8. Maltoni et al. (1988, 1977). In a study conducted at the Ramazzini Institute, Maltoni et al. (1988, 1977) exposed Sprague-Dawley rats (40/sex) to 5 mg/kg AN by gavage in olive oil, 3 days/week for 52 weeks; a control group of 75 rats/sex received olive oil alone on the same schedule (study designated BT203). After the exposure period, rats were maintained without further treatment for the rest of their natural lives (the study ending on week 131). Rats were examined 3 times daily for their general health status and were subjected to a clinical examination for gross changes every 2 weeks. Rats were weighed every 2 weeks during the first year and monthly thereafter. All rats were examined by gross necropsy. All tissues with gross lesions and a limited set of 12 tissues/organs from each rat were examined microscopically.

No statistically significant increases in tumors were observed in treated rats in this study; however, decreases in tumor latency or increased incidence were observed for some tumors types identified in other studies of AN. Papillomas and acanthomas of the forestomach were observed in 1/40 treated males (latency 92 weeks), 4/40 treated females (average latency 97.5 weeks), 0/40 control males, and 1/75 control females (latency 54 weeks). Maltoni et al. (1977) considered the increase in forestomach tumors to be treatment related. Gliomas (a category that includes astrocytomas) appeared in 1/40 treated females (latency of 33 weeks), 0/40 treated males, 2/75 control females (average latency 104 weeks), and 1/74 control males (latency 98 weeks). Tumors of the mammary gland and Zymbal gland were reported in treated animals, but the incidences were not elevated over the control. NTP recently released a memorandum (Malarkey et al., 2010) that discussed differences of opinion between NTP scientists and the Ramazzini Institute in the diagnoses of certain cancers reported in a methanol study conducted by the Ramazzini Institute. See Section 5.4.4.3 for additional information on the use of the Maltoni study in this assessment.

4.2.2.2.2. Maltoni et al. (1988, 1977). In studies conducted at the Ramazzini Institute, Maltoni et al. (1988, 1977) reported the results of three cancer bioassays in Sprague-Dawley rats exposed to AN by inhalation. In the first (designated BT201 by the authors), 30 rats/sex/group were exposed to 0, 5, 10, 20, and 40 ppm AN for 4 hours/day, 5 days/week for 52 weeks; the animals then were allowed to complete their natural life spans, with the final deaths occurring in week 136 (Maltoni et al., 1977). Rats were examined 3 times weekly for general health status and

subjected to a clinical examination for gross changes every 2 weeks. Rats were weighed every 2 weeks during the exposure period and monthly thereafter. All rats were subjected to gross necropsy. Histopathologic examinations were conducted on all gross lesions and a selection of about 12 organs and tissues, including the Zymbal glands, interscapular brown fat, salivary glands, tongue, lungs, liver, kidneys, spleen, stomach, intestine, bladder, and brain. Only the incidence of neoplastic lesions was reported.

Exposure to AN had no significant effect on survival or BWs in male or female rats. Increased incidences of gliomas, forestomach tumors, Zymbal gland carcinomas, and mammary tumors were reported in the treated group; however, increases in the incidences of these tumors in treated rats were not statistically significant. Gliomas were found in 1/30 and 2/30 males at 20 and 40 ppm, respectively, but not in controls and other exposed groups of male and female rats. The average latency of the gliomas was shorter at the higher concentration (84 weeks in 20-ppm males vs. 63.5 weeks in 40-ppm males). Forestomach papillomas and acanthomas were found in 0/30, 1/30, 2/30, 0/30 and 3/30 male rats at 0, 5, 10, 20, and 40 ppm, respectively. In females, the incidence of forestomach tumors was 0/30, 1/30, 2/30, 1/30, and 0/30 at 0, 5, 10, 20, and 40 ppm, respectively. The average latency of forestomach tumors ranged from 103 to 124 weeks. Zymbal gland carcinomas were found in 1/30 males in the 10-ppm group and 1/30 females in the 20-ppm group. No Zymbal gland carcinomas were found in the control and other dose groups. The incidence of benign and malignant mammary gland tumors in treated female rats was increased over controls, but the increase was not dose related (5/30 [controls], 10/30 [5 ppm], 7/30 [10 ppm], 10/30 [20 ppm], and 7/30 [40 ppm]) (Maltoni et al., 1977).¹

In two additional cancer bioassays, Maltoni et al. (1988) exposed Sprague-Dawley rats to AN by inhalation beginning in gestation. In the first bioassay (designated BT4003), 54 adult pregnant females, beginning on gestation day 12, were exposed to 60 ppm AN for 4 hours/day, 5 days/week for 7 weeks and then 7 hours/day, 5 days/week for 97 weeks. A group of 60 unexposed adult females served as controls. Gestation was permitted to proceed normally and the offspring were exposed on the same schedule as the dams. The exposed offspring included 67 males and 54 females; the controls included 158 males and 149 females. Overall, there was a statistically significant treatment-related increase in the percentage of dams with malignant tumors at all sites (37 vs. 15%). Increased incidences in exposed dams compared with controls were observed for several sites (Zymbal gland carcinomas, mammary gland

¹ Discrepancies were noted in the incidence of mammary gland tumors for controls and 40-ppm females as reported in a subsequent report of this study (Maltoni et al., 1988). In the 1988 publication, the incidence of mammary gland tumors was reported as 20% (6/30) in control females and 26.7% (8/30) in 40-ppm females. Also in the 1988 publication, encephalic gliomas were reported in both male and female rats at 20- and 40-ppm, whereas the 1977 publication reported gliomas in male rats only; glioma incidences reported in Maltoni et al. (1988) were: 20-ppm females—3.3% (1/30); 40-ppm females—3.3% (1/30); 20-ppm males—3.3% (1/30); 40-ppm males—6.7% (2/30)..

carcinomas, malignant mammary gland tumors, extrahepatic angiosarcomas, and encephalic gliomas), but none of these was statistically significantly different from controls. No hepatomas were observed in exposed or unexposed dams. These tumor results are summarized in Table 4-40.

Table 4-40. Comparison of carcinogenic effects of chronic exposure to AN at 60 ppm starting either in utero or in adulthood, in Sprague-Dawley rats

Stage during exposure	Exposure protocol	Sex ^a	Number of rats at start ^b	Percent with tumor				
				Brain tumors (encephalic gliomas)	Zymbal gland carcinomas	Hepatomas	Malignant mammary tumors	Extra-hepatic angio-sarcomas
Adult only	Chronic ^c	F	54	5.5	5.5	0.0	5.5	1.8
	Unexposed controls	F	60	0.0	1.7	0.0	3.3	0.0
Starting at GD 12	Chronic	M	67	16.4	14.9 ^c	7.5 ^d	0.0	4.4
		F	54	18.5	1.8	1.8	16.7 ^d	5.5 ^d
		M+F	121	17.3	9.1	4.9	7.4	4.9
	Subchronic ^e	M	60	5.0	6.7	1.7	0.0	5.0
		F	60	3.3	1.7	0.0	6.7	1.7
		M+F	120	4.2	4.2	0.8	3.3	3.3
	Unexposed controls	M	158	1.3	1.3	0.6	1.9	0.6
		F	149	1.3	0.0	0.0	5.4	0.0
		M+F	307	1.3	0.7	0.3	3.6	0.3

^aF = female; M = male.

^bAnimals were allowed to live until spontaneous death.

^cChronic: 60 ppm AN for 4 hrs/d, 5 d/wk for 7 wks (starting during gestation), followed by 7 hrs/d for 97 wks.

^dStatistically significantly higher than corresponding control incidence, $p < 0.05$.

^eSubchronic: 60 ppm AN for 4 hrs/d, 5 d/wk for 7 wks (starting during gestation), followed by 7 hrs/d for 8 wks.

Source: Maltoni et al. (1988).

In contrast, chronically exposed male and female offspring showed statistically significant increases in the incidences of malignant tumors of the mammary gland in females, extrahepatic angiosarcomas in females, hepatomas in males, Zymbal gland carcinomas in males, and encephalic gliomas (see Table 4-40).

In the second bioassay (designated BT4006), Sprague-Dawley rats were initially exposed to AN under the same exposure conditions as bioassay BT4003; however, exposure of the offspring (127 males and 114 females) ended after 15 weeks (Maltoni et al., 1988). This group of offspring was exposed for 4 hours/day, 5 days/week for 7 weeks starting on GD 12, followed by exposure for 7 hours/day, 5 days/week for 8 weeks. All animals were kept under observation until spontaneous death, at which time they were examined for the presence of tumors. The

control group of offspring was the one used in experiment BT4003 (158 males and 149 females). There was a statistically significant increase in the total incidence of malignant tumors in exposed offspring compared with controls for both males (31.7 vs. 17.1%, $p < 0.05$) and females (35.0 vs. 17.4%, $p < 0.01$). Increased incidences of the following tumors were observed when compared with controls, although the incidences were not statistically significantly different: Zymbal gland tumors in males and females combined; extrahepatic angiosarcomas in males; encephalic gliomas in males and females combined; and hepatomas in males (see Table 4-40).

NTP recently released a memorandum (Malarkey et al., 2010) that discussed differences of opinion between NTP scientists and the Ramazzini Institute in the diagnoses of certain cancers reported in a methanol study conducted by the Ramazzini Institute. See Section 5.4.4.3 for additional information on the use of the Maltoni et al. studies in this assessment.

5.4.4.3. Application of Age-Dependent Adjustment Factors (ADAFs)

AN is determined to be carcinogenic by a mutagenic mode of action (see Section 4.7.3.1), which raises concern for increased early-life susceptibility to cancer. Consistent with this possibility, two studies in Sprague-Dawley rats provide some evidence of increased cancer susceptibility associated with chronic AN exposure that begins in early periods of development—a chronic-duration inhalation cancer bioassay (Maltoni et al., 1988) and a three-generation drinking water reproductive toxicity study (Friedman and Beliles, 2002). According to the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (“*Supplemental Guidance*”) (U.S. EPA, 2005b), individuals exposed during early life to carcinogens with a mutagenic mode of action are assumed to be at increased risk for cancer. In these situations, the *Supplemental Guidance* recommends that age dependent adjustment factors (ADAFs) be applied in estimating cancer risk. The guidance further recommends that when data are available for a susceptible lifestage, those data should be used directly to evaluate risks for that chemical and that lifestage.

In the Maltoni et al. (1988) study conducted at the Ramazzini Institute, pregnant Sprague-Dawley rats were exposed to 60 ppm AN by inhalation for 104 weeks (see Section 4.2.2.2.2 for a complete study description). In addition, offspring of these dams were exposed to AN starting at GD 12 for a total of 104 weeks. Female rats exposed from GD 12 had a higher incidence of malignant mammary tumors, encephalic gliomas, and extrahepatic angiosarcomas than female rats exposed as adults. The drinking water bioassay by Friedman and Beliles (2002) involved 48-week exposures of each of three generations of female breeder Sprague-Dawley rats (see Section 4.2.1.2.9 for a complete study description). Compared with the 500-ppm F0 breeders that were exposed starting in adulthood, there was an increase in Zymbal gland and brain tumor incidence in the 500-ppm F1b female rats exposed starting in utero. Increases in tumor incidence

in the F2 generation were not statistically significantly different from F0 breeders.

The Maltoni et al. (1988) and Friedman and Beliles (2002) studies were considered for use in deriving chemical-specific ADAFs for early-life exposure to AN. The Maltoni et al. (1988) tumor results were not used to develop chemical-specific ADAFs because of concerns raised by a memorandum from NTP (Malarkey et al., 2010) that discussed differences of opinion between NTP scientists and the Ramazzini Institute in the diagnoses of certain cancers reported in a methanol study. While these data are considered qualitatively as support for early-life susceptibility, EPA decided not to rely on these data for quantitative purposes. The Friedman and Beliles (2002) study was also not considered further for the derivation of chemical-specific ADAFs. This study had several limitations, including a small number of animals per treatment group (20) and, therefore, limited power to detect tumor increases, and a lower tumor response in the F2b generation than the F1b generation. Therefore, although limited data are available supporting early-life susceptibility to carcinogenicity from AN exposure, the available information was not considered suitable for developing data-specific ADAFs. Accordingly, it is recommended that default ADAFs be applied to cancer risk values (OSF and IUR) for AN.

The *Supplemental Guidance* establishes default ADAFs for three specific age groups. These ADAFs and their corresponding age groups are: 10 for exposed individuals <2 years; 3 for exposed individuals 2 to <16 years; and 1 for exposed individuals \geq 16 years. The 10- and 3-fold adjustments are combined with age-specific exposure estimates when estimating cancer risks from early life (<16 years of age) exposures to AN. Example calculations for estimating cancer risks based on age at exposure are provided in Section 6 of the *Supplemental Guidance* (U.S. EPA, 2005b).

6.2.3. Oral Slope Factor

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Because a mutagenic mode of action for acrylonitrile carcinogenicity is sufficiently supported in laboratory animals and relevant to humans, and in the absence of adequate chemical-specific data to evaluate differences in susceptibility, increased early-life susceptibility is assumed. Accordingly, early-life susceptibility factors or ADAFs should be applied to the OSF when assessing cancer risk associated with early-life exposures (i.e., birth to 16 years) in accordance with the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b) (see Section 5.4.4.3).

6.2.4. Cancer Inhalation Unit Risk

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As described for the OSF, early-life susceptibility factors or ADAFs should be applied to the IUR when assessing cancer risks associated with early-life exposures (i.e., birth to 16 years) in accordance with the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b) (see Section 5.4.4.3).