Additional Toxicology Studies

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39. Several other studies have contributed to the understanding of the toxic effects of antimony across species and exposure durations.

40. In a study conducted by Kanisawa and Schroeder (1969), White Swiss mice of the Charles River Strain (CD-1) were exposed to 5 µg/ml antimony as potassium tartrate (equivalent to 350 µg Sb/kg bw/day in drinking water for life term. Compared to controls, no significant differences in the incidences of spontaneous tumours and malignant tumours were observed in the antimony treated group. 350 µg Sb/kg bw per day was concluded as NOAEL from this study.

41. In a lifetime exposure study conducted by Schroeder et al. (1970) on Long-Evans rats, animals were administered antimony potassium tartrate in drinking water at a dose of 430 µg Sb/kg bw/day. The study found a significant reduction in survival rates, with reduced non-fasting serum glucose levels. A Low Observed Adverse Effect Level (LOAEL) of 430 µg Sb/kg bw/day was identified based on these effects.

42. In the study conducted by Sunagawa (1981), groups of 5 Wistar rats were exposed to 0, 0.5, 1.0 or 2.0% metallic antimony in the diet (estimated doses of 0, 500,000, 1,000,000 and 2,000,000 µg Sb/kg bw/day) or 0, 1.0 or 2.0% antimony trioxide in the diet (0, 1,000,000 or 2,000,000 µg/kg bw/day corresponding to 0, 418,000, 836,000 µg Sb/kg bw/day) for 24 weeks. The description of this study from the Japanese literature is taken from the English language abstract. In the rats exposed to metallic antimony, significant adverse effects included dose-related decreases in body-weight gain, decreases in hematocrit and hemoglobin levels in the high-dose group and slight cloudy swelling in hepatic cords in the mid- and high-dose groups. Decreased erythrocyte levels and slight cloudy swelling of hepatic cords were observed in both groups of rats exposed to antimony trioxide. 418,000 µg Sb/kg bw/day was concluded as Low observed effect level (LOEL) dose from this study. The English abstract provided no further details on this study.

43. In a 12-week study conducted by Hiraoka (1986), groups of male Wistar rats (no information on number of animals per group) were treated with diets containing either 0.1% (w/w) of metal antimony (0.1%-Sb group, equivalent to 85,000 µg Sb/kg bw/day), 1.0% (w/w) of metal antimony (1.0%-Sb group, equivalent to 850,000 µg Sb/kg bw/day) or 1.0% (w/w) of antimony trioxide (1.0%-Sb_2O_3 group, equivalent to 700,000 µg Sb/kg bw/day). All the rats were allowed antimony-free diet for the following 12 weeks. Blood and organs were taken from the rats at the time of removal of the antimony-containing diet, 4 or 12 weeks after the removal of the antimony-containing diet. The results obtained were:

i. Neither abnormal behaviour nor unusual general appearance of the rats was observed in this experiment.

ii. The metal antimony and antimony trioxide inhibited the weight gain of rats. The weight of the rats of each 1.0%-Sb and 1.0%-Sb_2O_3 groups was lighter than that of 0.1%-Sb group. During recovery, the rats increased in weight up to the normal level at 12 weeks after removal of the antimony-containing diet.

iii. The haematocrit in blood from 1.0%-Sb group rats was significantly decreased at 4 weeks after the removal of the antimony-containing diet. The total protein levels in blood from 1.0%-Sb group rats was significantly decreased at the time of removal of the antimony-containing diet. A significant increase of alanine transaminase (ALT - reported as Glutamate Pyruvate Transaminase (GPT)) level was seen in the blood from 0.1%-Sb group rats at 4 weeks after the supply of the antimony-free diet began. No significant changes of Hb and aspartate transaminase (AST – reported as glutamic-oxaloacetic transaminase (GOT)) levels and albumin to globulin (A/G) ratio were found in the blood samples from all rats.

iv. Some significant changes of the organ weight and the ratio between organ weight and body weight of the rats, after the administration of Sb and Sb2O3 containing diet, were found.

v. High concentrations of antimony were found in liver, spleen, lungs, hairs and bone and the highest concentration was detected in the blood of the rats. A NOAEL of 700,000 µg Sb/kg bw/day was identified for antimony trioxide.

44. In a study by Rossi et al. (1987), pregnant female rats were exposed to antimony trichloride (1 and 10 mg/L in their drinking water ad libitum equivalent to 70 µg Sb/kg bw/day and 700 µg Sb/kg bw/day, respectively) from the first day of pregnancy until weaning (22nd day after delivery). Pups were exposed to antimony trichloride (1 and 10 mg/L in their drinking water ad libitum) from 22nd until 60th day of age. Antimony exposure did not significantly affect maternal and pup systolic arterial blood pressure, length of gestation or number of newborns per litter. Maternal body weight decreased significantly in a dose-dependent manner by the 20th day of gestation at both 0.1 and 1 mg/dl doses. Pups at high dose showed significantly reduced body weight from the 10th to the 60th day after birth. A NOAEL of 70 µg Sb/kg bw/day and a LOAEL of 700 µg Sb/kg bw/day were concluded based on decreased maternal body weight gain (11%) and decreased pup growth on postnatal days (PNDs) 10–60.

45. In a 14-day study conducted by NTP (1992), groups of 10 male and 10 female B6C3F1 mice were exposed to 0, 300, 650, 1,250, 2,500, 5,000 mg/L antimony potassium tartrate (99–100% purity) in drinking water. The investigators used water consumption data and body weight averages to calculate doses of 0, 59,000, 98,000, 174,000, 273,000 and 407,000 µg/kg bw/day antimony potassium tartrate (0, 21,000, 36,000, 63,000, 99,000 and 150,000 µg Sb/kg bw/day).

46. The following parameters were evaluated to assess toxicity: twice daily observations, body weight measurements (days 1 and 8 and at termination), water consumption (days 7 or 8 and day 15), organ weights, histopathology of major tissues and organs in control and high-dose groups (five mice/sex/group) and all early deaths and histopathological examination of the liver and forestomach of mice in all groups (five mice/sex/group). One female mouse in the 150,000 µg Sb/kg bw/day group died prior to the end of the study. On day 8, decreases in body weight gain were observed in males exposed to 99,000 µg Sb/kg bw/day and in males and females exposed to 150,000 µg Sb/kg bw/day. However, by the end of the study, the final weights of all antimony groups were within 93% of the controls. Decreases in water consumption were observed at all antimony levels.

47. The investigators noted that overt signs of toxicity (rough haircoat, emaciation, abnormal posture, hypoactivity and decreased faecal material, consistent with avoidance of the antimony potassium tartrate containing water) were observed, but did not specify if this was observed in all groups. Histological alterations were observed in the forestomach and liver of mice in the 150,000 µg/kg bw/day group. In the forestomach, focal areas of ulceration with necrosis and inflammation of the squamous mucosa were observed; the incidence was not reported, although the investigators noted that gross forestomach lesions were observed in one male and three females. In the liver, minimal to moderate cytoplasmic vacuolization was observed in all mice in the 150,000 µg Sb/kg bw/day group; the vacuolization had a centrilobular distribution with some extension into portal areas. The NOAEL for liver toxicity was determined to be 99,000 µg Sb/kg bw/day.

48. As part of NTP (1992) study, groups of F344/N rats were administered APT in drinking water for 14 days. The animals were assigned to dose groups of 10 rats/sex. Drinking water doses, estimated by water consumption, were 0, 16,000, 28,000, 59,000, 94,000, or 168,000 µg/kg (0, 5,800, 10,000, 21,000, 34,000, 61,000 µg Sb/kg bw/day). APT was poorly absorbed and relatively nontoxic when given orally. There was no mortality or histopathological lesions in rats receiving doses of APT as high as 168,000 µg/kg. 61,000 µg Sb/kg bw/day was concluded as NOAEL dose from this study.

49. A 90-day dietary study of antimony trioxide was conducted in male and female Wistar rats (Alpk:APSD strain) by Hext et al. (1999). Rats (12/sex/group) were fed diets containing 0, 1,000, 5,000 or 20,000 ppm antimony trioxide (99% purity) resulting in doses for the male rats of 0, 70,000, 353,000, 1,408,000 µg

Sb/kg/day and for female rats of 0, 81,000, 413,000, 1,570,000 µg Sb/kg/day. Food consumption was measured continuously and calculated as a weekly mean. Body weights were measured weekly. Doses were calculated for each week, based on feed consumption and body weight. Cage-side observations were made daily and detailed clinical observations were made weekly. During the last week of the study, control and high-dose rats received an eye examination using an indirect ophthalmoscope and a mydriatic substance to dilate the pupil. Urine samples were collected (16-hour collection) from rats housed in metabolic cages during the last week of the study. Urine volume was measured, and samples were analysed for appearance, specific gravity, pH, glucose, ketones, bilirubin, protein and blood. Urine was centrifuged and the sediment was stained and examined. Blood samples were obtained for haematology and clinical chemistry by cardiac puncture following sacrifice by halothane overdose. Adrenal glands, brain, kidneys, liver, epididymides and testes were removed, weighed and prepared for histopathological examination. All tissues from the control and high dose rats were examined, as well as any abnormal tissue from the intermediate dose groups.

50. Food consumption and body weight gain were similar to controls for all treatment groups. No significant clinical signs or ocular changes were associated with exposure to antimony trioxide. In high-dose female rats, urine volume was increased (+79%) and specific gravity was decreased (-1%). Urinary pH was increased in male rats given 1,000 ppm (+5%) or 20,000 ppm (+5%) but was similar to the control value in the 5,000 ppm group. Changes in urinary parameters were not dose-related and were considered by the study authors to be incidental.

51. Minor changes were noted in some haematological parameters, with an elevated red cell count in high-dose male rats (+4%) and a decreased mean cell volume in high-dose female rats (-2%). The study authors considered the haematological changes to be too small to be of toxicological significance. Triglyceride content was increased (+30%) and alkaline phosphatase activity was decreased (-12%) in high-dose male rats. High-dose female rats exhibited an increase in plasma cholesterol (+13%), a decrease in alkaline phosphatase activity (-36%) and an increase in aspartate aminotransferase activity (+52%). Alkaline phosphatase activity was also decreased (-23%) in female rats given 5,000 ppm of antimony trioxide in the diet. No other treatment related changes in plasma biochemistry were observed. Absolute and relative liver weights were increased by approximately 10% in female rats fed 20,000 ppm antimony trioxide. No gross findings indicative of toxicity was seen at necropsy. The

incidence of pituitary cysts was higher in the 20,000 ppm dose groups of both male and female rats (4/12 treated males, 3/12 treated females, 1/12 control males and females). The study authors considered pituitary cysts to be a common spontaneous lesion with reported incidence values within the historical control range (i.e., not treatment-related). Three male rats in the high dose group had slight to moderate plasma cell infiltration in the cervical lymph node. This change has also been previously seen in historical controls from the same laboratory and was therefore not considered treatment related. No other histopathological lesions were observed.

52. Considering small increase in liver weight, small decrease in plasma alkaline phosphatase activity and small increase in plasma aspartate and alanine aminotransferase levels in the high dose group without any histological correlate in the liver. The high dose was concluded to be the NOAEL equivalent to 1,408,000 µg Sb/kg bw/day (male rats) and 1,570,000 µg Sb/kg bw/day (female rats).

53. In a study conducted by Omura et al. (2002), the testicular toxicity of antimony trioxide was evaluated in Crj:Wistar rats (7-8/group) and Cjr:CD-1 mice (8-10/group). Antimony trioxide (purity >99.9%) (12,000 or 1,200,000 µg/kg-day) was administered by oral gavage to rats (3 days/week for 4 weeks) and mice (5 days/week for 4 weeks). Animals were sacrificed by carbon dioxide inhalation 24 hours after the final gavage dose was administered. The testes, epididymides, ventral prostate and seminal vesicle (without fluid) were removed and weighed. Histopathological changes were evaluated in the testes and the number, motility and morphology of sperm from the cauda epididymides were assessed. Three mice (1 control, 2 given 1,200,000 µg/kg-day) died due to gavage error. No significant effect on body weight or organ weight of reproductive tissues was observed. Sperm parameters were not affected by antimony trioxide treatment and histopathology results were essentially negative. A NOAEL of 1,200,000 µg/kg/day (highest dose tested) was concluded for male reproductive effects of antimony trioxide in this study.

54. In a REACH registration dossier submitted to ECHA, pregnant female Sprague-Dawley rats received sodium hexahydroxoantimonate via gavage at the dose levels of 0, 49,000, 148,000, 493,000 µg Sb/kg bw per day between gestation day 6-19. Increased (non-significant) incidence in delayed skeletal development were observed in the mid and high dose groups. Most values were only slightly above historical control data. When considering skeletal malformations overall, incidence was observed in 99.3% to 100% of fetuses and 100% of litters including

controls. 49,000 µg Sb/kg bw per day was concluded as NOAEL from this study.

55. Table 1 below summarises all the NOAEL values concluded from the available studies, as well as those concluded by authoritative bodies.

Table 1 NOAELs from studies and concluded by authoritative bodies

Authority or Authors	**NOAEL (µg Sb/kg bw/day)**	**Critical effects**	**Study Reference**
World Health Organization (WHO)	6,000	Observed effects at lower doses considered adaptive and not of toxicological significance.	Poon et al. (1998) Lynch et al. (1999)
ATSDR	60	Decreases in serum glucose levels.	Poon et al. (1998)
Health Canada	60	Histological liver changes, alterations in serum biochemistr.	Poon et al. (1998)
Kanisawa & Schroeder (1969)	350	No significant differences in spontaneous and malignant tumour incidence in mice.	Kanisawa & Schroeder, 1969
Schroeder et al. (1970)	430 (LOAEL)	Reduced survival rate in males and females; at the median life spans, survival was reduced by 106 and 107 days for males and females, respectively, compared to controls. Non-fasting serum glucose levels were reduced by 28%–30% in the dosed animals.	Schroeder et al. (1970)

Authority or Authors	NOAEL (µg Sb/kg bw/day)	Critical effects	Study Reference
Sunagawa (1981)	418,000	Liver histopathological changes and increased aspartate transaminase (AST) activity.	Sunagawa, 1981
Hiraoka (1986)	700,000	Inhibited weight gain, changes in hematocrit and protein levels, high antimony concentrations in organs	Hiraoka, 1986
Rossi et al. (1987)	70	Decreased maternal body weight gain and decreased pup growth on PNDs 10–60.	Rossi et al. (1987)
NTP (1992)	99,000	Forestomach lesions in the high dose group. Dose-related increases in relative liver weight; lesions in the liver of most mice in the high dose group.	NTP (1992)
NTP (1992)	61,000	Increase in relative liver weight in the high dose group	NTP (1992)
Hext et al. (1999)	1,408,000 males), 1,570,000 (females)	Small increase in liver weight, small decrease in plasma alkaline phosphatase activity and small increase in plasma aspartate and alanine aminotransferase levels in the high dose group. No histological effects on liver.	Hext et al. (1999)
Omura et al. (2002)	1,200,000	No significant effect on reproductive organs or sperm parameters.	Omura et al. (2002)

Authority or Authors	NOAEL (µg Sb/kg bw/day)	Critical effects	Study Reference
ECHA (2014)	49,000	Increased (non-significant) incidence in delayed skeletal development in the mid and high dose groups. Most values were only slightly above historical control data. When considering skeletal malformations overall, incidence was observed in 99.3% to 100% of fetuses and 100% of litters including controls. No reproductive toxicity, embryotoxicity or fetotoxicity.	Dossier submitted to ECHA (2014)