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i2a's assessment of NTP's long-term carcinogenicity studies on antimony trioxide (ATO)

Introductory summary

The profile of substances such as antimony trioxide may at first seem to be relatively simple and straight forward. However, few things are not as simple as they may at first seem:

- There are several reports of lung tumors after inhalation exposure of rats, with NTP recently finding some evidence for increased incidence of pulmonary neoplasms. However, impacts in the rat are difficult to interpret in light of evidence of the pulmonary overload known to occur in many studies. In the recent NTP inhalation studies, indications of pulmonary overload are acknowledged at the 10 and 30 mg/m^3 exposure levels. Indeed, even at 3 mg/m^3 clearance deviates from modelled predictions towards the end of the study in a fashion that one would expect under overload. Interpretation of lesions that develop under conditions of pulmonary overload is presently the subject of much debate and transcends the substance-specific effects of antimony. An increased incidence of pheochromocytomas was also observed in rats. There is a significant body of evidence that such lesions are expected under conditions of pulmonary inflammation and hypoxia. They are a response to substance-induced pulmonary impacts and not a response to the substance itself, which is confirmed by the clinical observations that indicated that rats (at 10 and 30 mg/m^3) and all exposed mice were suffering from abnormal breathing and thinness. Indeed, combined with significant body weight depression associated with inhalation exposure of rats and mice to antimony trioxide, these data suggest that the NTP study of rats was conducted at excessive airborne concentrations of antimony trioxide that inappropriately exceeded the maximum tolerated dose.

- The recent NTP studies of inhalation imparts in mice at first seem to provide solid evidence of carcinogenicity, but even this study is surrounded by a host of questions and uncertainties. If one adopts a critical eye, all exposure levels in this study produced significant pulmonary inflammation and sufficient impairment of pulmonary function to yield systemic hypoxia. Under the conditions of local pulmonary toxicity and systemic hypoxic stress, increases in neoplastic lesions, particularly those with high spontaneous incidence (such as B-cell lymphomas especially in female mice), must be interpreted with caution.

- As stressed above, any increase in B-cell lymphomas must be interpreted with caution. The histological work-up of these lesions by NTP was not informative and does not permit detailed diagnostic classification. Are these reactive lesions arising in response to pulmonary inflammation and hypoxia? Quite possibly so. Female B6C3F1N mice have a high background of spontaneously arising B cell lymphomas and increases are difficult to interpret. This is in contrast to T cell lymphomas that are most often chemically induced.

- Increased incidence of lung tumors in mice? A seemingly solid finding until one considers the high spontaneous incidence of lesions in the mouse lung and asks what might be expected under condition of severe inflammation and toxicity. Are the observed lesions chemically induced or are they, a response to altered conditions in the lung that permit enhanced clonal expansion of spontaneous lesions?

- The genotoxicity data do not help us answer this:

- *In vitro* studies show responses at high concentrations that appear to entail indirect mechanisms such as induction of reactive oxygen species or inhibition of DNA repair.

- NTP reported a very modest increase in erythrocyte micronuclei – but such increases are associated with perturbations in erythropoiesis similar to those we know occurred in mice.

- Positive Comet assay are reported by NTP, but such data needed to be adequately controlled for cytotoxicity and apoptosis. This is especially true given the conditions known to exist in the mouse lungs.

- Appearance of tumors with EGFR activated oncogenes? Past NTP studies have reported chemical specific fingerprints for the mutations seen in activated oncogenes, but oncogene activation via generic point mutations similar to spontaneous lesions are more difficult to interpret. The human experience tells us that patterns of oncogene activation in response to a single agent (cigarette smoke) vary as a function of time to tumor incidence, most likely as a function of selection for different tumor phenotypes. A small but growing body of evidence indicates that EGFR lesions confer a proliferative advantage to neoplasms growing under hypoxic conditions.

When all is said and done – yes we know the tumors developed after inhalation exposure to antimony trioxide. We know that some are likely arising in response to the hypoxic effects created by exposure to antimony trioxide and not as a direct result of exposure to antimony trioxide per se. Others could be specifically induced by antimony trioxide, but at this point we do not know how or why.

In light of the above, i2a is embarking upon a program of research to address fundamental issues and generate missing information pertinent to uncertainties associated with the carcinogenic and mutagenic properties of antimony and its compounds.