



Experimental Pathology Laboratories, Inc.

INDUCTION OF TUNICA VAGINALIS MESOTHELIOMAS
IN RATS BY XENOBIOTICS

EPL PROJECT NO.: 856-001

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EXECUTIVE SUMMARY

This white paper provides an overview of the basic biology, key events, mode of action, and examples of xenobiotics associated with tunica vaginalis mesotheliomas reported in rat cancer bioassays. The goal is to better understand the relevance of rat tunica vaginalis mesothelioma bioassay responses to human carcinogenesis.

Spontaneous tunica vaginalis mesotheliomas are rare in all species, are seen in older animals, and most commonly occur in male F344 rats at the end of two-year studies. They arise from the mesothelial lining of the scrotal sac, testis, and epididymis, rarely metastasize in rats, but may spread by extension or seeding into the contiguous peritoneal cavity. Mesotheliomas in the peritoneal cavity of rats invariably are also present in the tunica vaginalis, their site of origin. Unlike asbestos-induced pleural mesotheliomas, tunica vaginalis mesotheliomas in the F344 rat are generally less invasive, less anaplastic, and have minimal stromal components. As with most well studied cancers, a spectrum of lesion severity ranging from hyperplasia to benign neoplasia, and ultimately to malignant neoplasia, is characteristic of tunica vaginalis and peritoneal mesotheliomas in rats.

Mesothelial cells are relatively easy to culture *in vitro* where they can undergo spontaneous as well as treatment-induced transformation to gain malignant phenotypes. Likewise, cells derived from spontaneous and fiber-induced mesotheliomas have been cultured. *In vitro* studies with transformed mesothelial cells and primary mesothelioma cultures have allowed identification of early response genes, tumor suppressor genes, and several growth factors that drive the cell proliferation process and possibly impair anti-tumor host defenses. Growth factors elaborated both by stimulated mesothelial cells and mesothelioma cells function in an autocrine fashion to stimulate their own proliferation.

Hormone imbalance brought about by perturbations of the endocrine system has been proposed as a key factor leading to both spontaneous and treatment-associated tunica vaginalis mesotheliomas in rats. Age-associated hormonal changes are especially dramatic in F344 rats and these changes, particularly the leutinizing hormone response to age-associated decreased

testosterone, are causally related to the nearly 100% incidence of Leydig cell tumors seen in the untreated male F344 rat at the end of two-year studies. Once established, the multiple Leydig cell tumors cause enlargement of the testes and alter the intratesticular hormonal balance. The altered testicular hormonal milieu is reflected as a transudate that bathes the mesothelial lining of the scrotal sac and stimulates the release of autocrine growth factors that lead to mesothelial proliferation and ultimately tunica vaginalis tumors. Additionally, the enlarged testes exert pressure on the tunica vaginalis mesothelium which also stimulates the elaboration of mesothelial autocrine growth factors.

All but 2 of the 21 published rat cancer bioassays with a treatment-associated tunica vaginalis mesothelioma response were carried out in F344 rats. Examination of the mesothelioma data allowed categorization of the responses as either robust or marginal/non-significant based primarily on the magnitude of the mesothelioma response. Nine of the 21 studies were judged to have a non-significant to marginal response, i.e., TVM frequency <25%, an additional 3 with low frequencies are relatively non-informative because of early study termination due to other induced tumors, and 9 exhibited a robust response based on a greater than a 25% maximum incidence of mesotheliomas. Of the 9 chemicals producing a robust mesothelioma response to treatment, 7 were mutagenic in Salmonella. Where *in vitro* cytogenetics results were available, they supported the Salmonella results. Only 3 of the 9 studies with non-significant to marginal responses were mutagenic in Salmonella.

Based on published literature and the studies reviewed in this white paper, the tunica vaginalis mesothelioma response is unique to the male F344 rat. Excluding three studies with direct contact to the mesothelium because of intraperitoneal administration, 18 studies included female F344 rats and 10 included male and female B6C3F1 mice. Mesotheliomas were not observed in female rats or either gender of mice, even for those agents with a robust mesothelioma incidence in male rats. Furthermore, in the studies that were not terminated prematurely in which other testicular lesions were reported, all had a Leydig cell tumor incidence of greater than 80%.

An autocrine growth factor-driven mode of action is believed to be a primary cause of the observed tunica vaginalis mesothelioma responses seen in the 9 studies with a non-significant to marginal response.

The primary conclusions based upon this review of tunica vaginalis mesotheliomas in rat bioassays are as follows:

- Tunica vaginalis mesotheliomas are low-incidence spontaneous neoplasms in rats that can be exacerbated by chemical treatment. They are most common in F344 males.
- The mesothelioma responses to xenobiotic exposure are male F344 rat-specific. They are not seen in female F344 rats or in either gender of mice in conventional, 2-year, cancer bioassays.
- Tunica vaginalis mesotheliomas in rats originate in the mesothelial lining of the scrotal sac, testes, epididymides, and mesorchium, and can spread to the abdominal cavity by extension or seeding because the scrotal sac mesothelium is continuous with the peritoneal cavity mesothelium in the rat.
- Spontaneous, as well as several xenobiotic-associated tunica vaginalis mesotheliomas, are causally associated with Leydig cell tumors which lead to an autocrine growth factor-induced mesothelial mitogenesis.
- Most of the chemicals that induce a robust tunica vaginalis mesothelioma are mutagenic in the Ames test, whereas the majority of chemicals that induce a non-significant to marginal response are not.
- Based on the male F344 rat specificity of tunica vaginalis mesotheliomas, the causative association with the F344 Leydig cell tumor burden, and the lack of genotoxicity for the majority of chemicals associated with a marginal mesothelioma response, a non-significant to marginal tunica vaginalis mesothelioma response is not relevant to humans, and does not pose a risk for human cancer.

INTRODUCTION

Spontaneously occurring mesotheliomas have been documented in a wide range of animals but are relatively rare. They have been observed in humans, lower vertebrates, domesticated and laboratory reared mammals, avian species, and marsupials, and occur in the thoracic and abdominal cavities (Ilgren, 1993; Crosby, 2000; Crosby et al., 2000) with rare reports of atriocaval mesotheliomas in cardiac chambers (Hoch-Ligeti et al., 1986; Peano et al., 1998; Chandra et al., 1993). Spontaneous mesotheliomas, which occur primarily in the scrotal sac and peritoneal cavity, have been documented in various rat strains, with the highest frequency

Table 30. Genetic toxicity of chemicals associated with tunica vaginalis mesothelioma induction in rats, arranged in order of decreasing tumor frequency*

Carcinogen	CASRN	TVM frequency ^a	Ames test	In vitro cyto	In vivo (bone marrow) cyto
Robust TVM induction					
<i>o</i> -Nitrotoluene	88-72-2	90%	–	–	–
Glycidol	556-52-5	83%	+	+	+
Cytembena (ip)	21739-91-3	74%	+	+	–
Nitrilotriacetic acid ± ferric saccharate (ip)		68%	**		
Potassium bromate	7758-01-2	63%	+		
1,2-Dibromoethane	106-93-4	52%	+	+	–
Methyl (acetoxymethyl)nitrosoamine (ip)	56856-83-8	46%	+		
2,2-Bis(bromomethyl)-1,3-propanediol	3296-90-0	43%	+	+	E
<i>o</i> -Toluidine HCl	636-21-5; 95-53-4 ^b	34%	+	+	+/-
Non-significant-to-marginal TVM induction					
Methyleugenol	93-15-2	24%	–	–	–
Pentachlorophenol, purified	87-86-5	18%	–	w+	–
Acrylamide	79-06-1	17%	–	+	w+
Ethyl tellurac	20941-65-5	16%	–	E	
Nitrofurazone	59-87-0	14%	+	+	–
Ethylene oxide	75-21-8	13%	+	+	+
Tartrazine	1934-21-0	12%	–	+	
Benzaldehyde	100-52-7	10%	–	–	
1,2-Dichloroethane	107-06-2	10%	+	+	–

* All genetic toxicity results are from the NTP database except ethylene oxide. Three studies (3,3'-dimethylbenzidine HCl, 3,3'-dimethoxybenzidine HCl, 2,3-dibromo-1-propanol) that terminated early are not listed.

**Nitrilotriacetic acid is not mutagenic in the Ames test; there are no data available on the mutagenicity of ferric saccharate or the combination.

a. % of treated animals with TVM; maximum response recorded

b. combined results from testing different salts of the same parent chemical

ip, intraperitoneal administration; +, positive response; w+, weakly positive; –, negative; E, equivocal response; +/-, conflicting results; blank, not tested

Table 31. Human relevance framework for tunica vaginalis mesothelioma induction in F344 rats secondary to enhanced mesothelial mitogenesis

Alternative Key Events	Degree of Certainty in F344 Rat	Human Relevance
Presence of Leydig cell tumors causally related to tunica vaginalis mesotheliomas	Reasonably certain. Size of Leydig cell tumors correlated with tunica vaginalis mesotheliomas and localized growth factors. Localized peritesticular hormonal imbalance stimulates mitogenic autocrine growth factors from mesothelial cells. (Turek & Desjardins 1979; Gerwin et al., 1987; Karpe et al., 1982; Bartke et al., 1985; Versnel et al., 1988)	Not relevant. Leydig cell tumors are extremely rare in humans. There are significant differences in production and responsiveness between rat and human mesothelial cells. (Clegg et al., 1997; Walker et al., 1995; Walker et al., 1992)
Physical pressure or shearing forces due to enlarged Leydig cell tumor-bearing testes	Good evidence. Evidence for altered growth factor expression in transformed mesothelial cells in vitro. (Tanigawa et al., 1987; Gabrielson et al., 1988; Gerwin et al., 1987; Waters et al., 1997)	Not relevant. Leydig cell tumors are extremely rare in humans. (Clegg et al., 1997; Walker et al., 1995; Walker et al., 1992)
Age-associated increased prolactin leading to decreased circulating testosterone	Certain. Increased prolactin causes decreased LHRH and LH and inhibition of testosterone production. (Mahoney & Hodgen 1995; Capen et al., 2002)	Not relevant. Human Leydig cells do not have LHRH receptors. LH receptors not responsive to prolactin. (Prentice & Meikle 1995)
Decreased prolactin secretion from pituitary via dopamine agonists	Certain for specific chemicals. Serum prolactin levels decrease in rats. Decrease in LH receptors. (Prentice et al., 1992; Prentice & Meikle 1995; Friedman et al., 1999; Uphouse et al., 1982)	Not relevant. Human LH receptors not responsive to prolactin. (Wahlstrom et al., 1983)
Spontaneous age-associated decrease in testosterone and LH receptors and compensatory increase in LH	Certain. Responsible for the high spontaneous incidence of Leydig cell tumors in older F344 rats. (Amador et al., 1985; Maekawa & Hayashi 1992; Takaki et al., 1989; Solleveld et al., 1984; Foster 2007; Tanigawa et al., 1987; Turek & Desjardins 1979; Prentice & Meikle 1995; Capen 1996)	Uncertain. The number of LH receptors is 14 times greater in rats compared to humans. (Prentice and Meikle, 1995)
LHRH receptor agonist induced Leydig cell tumors	Reasonably certain for specific chemicals. Binding to rat LHRH receptors on Leydig cells produces Leydig cell tumors. (Prentice & Meikle 1995)	Not relevant. Human Leydig cells do not have LHRH receptors. (Prentice & Meikle 1995)