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The Use of the Tg.rasH2 Mouse in Carcinogenicity Studies

As reported in our Fall 2007 Newsletter a 26-week carcinogenicity validation study in Tg.rasH2 mice was initiated at ITR Laboratories Canada Inc. in June of 2007 and is now completed.

The objective of this study was to evaluate the response of the Tg.rasH2 mouse test system to two known carcinogens and to demonstrate its utility as a quicker and more cost-effective alternative to the conventional two-year mouse bioassay.

Tg.rasH2 mice were assigned to each of 3 dose groups as follows:

Group Number And Designation	Test/Control articles	Dose Level	Dose Conc.	Dose Volume	Number of Animals	
		(mg/kg)	(mg/mL)	(mL/kg)	Male	Female
1. Control	Purified water	0	0	10	15	15
2. Test Article	Urethane	1000	100	10	15	15
3. Test Article	N-methyl-N-nitrosourea	75	7.5	10	15	15

Mice of Group 1 received purified water by oral gavage daily for 26 weeks. Animals of Group 2 received 3 intraperitoneal injections of Urethane (1000 mg/kg each) at 2-day intervals (on Days 1, 3 and 5), while animals of Group 3 received a single intraperitoneal injection of MNU (75 mg/kg) on Day 1.

Parameters monitored during the study included mortality, clinical observations, including examinations for the presence of palpable masses, body weights and food consumption. Upon completion of the 26-week study period, all surviving animals were euthanized and subjected to a necropsy examination. Subsequently, tissues collected from all animals were examined histopathologically.

The cause of death/preterminal euthanasia within each of the dose groups is summarized as follows:

Group / sex	1M	2M	3M	1F	2F	3F
No. of animals killed or dying	0	15	12	2	13	11
Blood vessel tumor	0	12	2	1	5	1
Lung tumor	0	3	0	1	6	0
Malignant lymphoma	0	0	8	0	0	9
Subcutaneous tumor	0	0	1	0	0	0
Stomach tumor	0	0	1	0	1	0
Unclear	0	0	0	0	1	1

A total of 2 Control, 28 Urethane-treated and 23 MNU-treated animals died or were preterminally euthanized due to poor clinical condition during the study. The deaths and preterminal sacrifices occurred between Days 39 and 173 of the study.

Bronchiolo-alveolar carcinoma of the lungs and hemangiosarcoma of the spleen were considered to be the cause of mortality/morbidity of the two Control females.

Microscopic examinations performed on tissues obtained from the preterminally euthanized/found dead Urethane-treated animals revealed a high incidence of bronchiolo-alveolar adenoma and/or carcinoma and hemangiosarcoma. These findings were considered to be the cause of mortality/morbidity in the majority (26/28) of these animals

Microscopic examinations performed on tissues obtained from the preterminally euthanized/found dead MNU-treated animals revealed a high incidence of malignant lymphoma of the hemolymphoreticular tissue. These findings were considered to be the cause of mortality/morbidity in the in the majority (17/23) of these animals, while hemangiocarcinoma was considered to be the second most frequent tumor resulting in the death of 3/23 animals.

In conclusion, the intraperitoneal administration of 2 known carcinogens (Urethane and MNU) to Tg.rasH2 mice followed by a 26-week holding period produced a clearly higher incidence of benign/malignant tumors and tumor bearers resulting in significantly higher mortality rates among the animals treated with the carcinogens (Urethane and MNU) in comparison to Controls. Consequently, the use of the Tg.rasH2 mouse model for carcinogenicity studies is now validated at ITR Laboratories Canada Inc. as a quicker and more cost-effective alternative to the conventional two-year mouse bioassay.

NEWS:

FDA Inspection:

At the beginning of July 2008 ITR was inspected by a team of 3 staff from the FDA who spent 5 days at the laboratory viewing procedures, auditing the facility, and closely scrutinizing the data from two 28 day studies. Only 4 findings were present on the 483; 2 of these findings pertained to the draft status of the study reports reviewed and were resolved by finalisation; another finding related to a computer system that is no longer in use following our introduction of Provantis, and the final finding simply required a modification to a process that was immediately implemented.

Provantis Update:

We are pleased to confirm completion of the validation of Provantis for in-life, clinical pathology and necropsy/pathology areas. Provantis is now being used exclusively to collect and report data in these areas.

Upcoming Events:

ITR will have representatives at the following conferences and look forward to seeing you there:

- **BioContract Quebec** in Quebec City on October 1-3
- **Association of Inhalation Toxicology** in Dublin on October 8-10
- **ACT** in Tucson, Arizona on November 9-12