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A Collaborative Validation Study for the Gpt Delta Mouse Using N-propyl-N-nitrosourea, Diethylnitrosamine, Mitomycin C and Chlorambucil: A Summary Report of the Third Collaborative Study of the Transgenic Mouse Mutation Assay by JEMS/MMS

Nobuhiro Yajima[1*,§], Atsushi Hyogo[2], Hironobu Tamura[3], Madoka Nakajima[4] and Takehiko Nohmi[5]

[1]*Research Institute of Life Science, Snow Brand Milk Products Co., Ltd., 519, Shimo-ishibashi, Ishibashi-machi, Shimotsuga-gun, Tochigi, 329-0512, Japan*

[2]*Medicinal Safety Research Laboratories, Sankyo Co., Ltd., 717, Horikoshi, Fukuroi-shi, Shizuoka 437-0065, Japan*

[3]*Nippon Shinyaku Co., Ltd., Safety Laboratory, Kisshouin 14, Minami-ku, Kyoto, 601-8550, Japan*

[4]*Biosafety Research (An-pyo) Center, Foods, Drugs & Pesticides, 582-2, Arahama Shioshinden, Fukude-cho, Iwata-gun, Shizuoka 437-1213, Japan*

[5]*Division of Genetics and Mutagenesis, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan*

To validate a novel mouse model, *gpt* delta, for in vivo mutagenesis, the Mammalian Mutagenesis Society (MMS), a subgroup of the Environmental Mutagen Society of Japan (JEMS) (JEMS/MMS), performed a collaborative study as the third trial for transgenic animal assay. In this mouse model, point mutations and deletions are separately identified by *gpt* (6-thioguanine-resistant) and Spi- (sensitive to P2 interference) selections, respectively. A preliminary study made a rule that acceptable total number of colonies for *gpt* selection was higher than 0.5×10^6 colony forming units/tissue/animal and that of plaques for Spi- selection was higher than 1.0×10^6 plaque forming units/tissue/animal, respectively. In the main study, the mice were once intraperitoneally injected *N*-propyl-*N*-nitrosourea (PNU; an alkylating agent), diethylnitrosamine (DEN; a promutagen), mitomycin C (MMC: a cross-linking agent) or chlorambucil (CB; a lung carcinogen in laboratory animal) and the mutant frequencies (MFs) were determined. *gpt* MFs increased in the bone marrow, liver and spleen of PNU-treated mice on Day 28. *gpt*

MF in the bone marrow showed the maximum value on Day 3 and then decreased to constant over the following Days 7, 14 and 28 while Spi⁻ MF seemed to be slightly increased until Day 14 following obvious increase on Day 3, and was further decreased on Day 28. A remarkable increase in *gpt* MFs was observed in the liver of DEN-treated mice on Day 28 after the treatment, although no induction of Spi⁻ mutations was observed. On the contrary, only one treatment with MMC efficiently induced Spi⁻ MFs in the bone marrow and liver, but not testis, on Days 3, 7, 14 and 28. In CB-treated mice, dose-related increases in *gpt* MFs were observed in the liver and lung on Day 28, while no induction of Spi⁻ mutations was observed in the liver, lung, spleen or testis. These results are consistent with the expected spectra of mutations detectable by the *gpt* assay and Spi⁻ selection, and thus raise the possibility that the Spi⁻ assay can be used as an alternative method for in vivo micronucleus testing.

Keywords : chlorambucil; diethylnitrosamine; gpt (6-thioguanine-resistant; 6-TGr) selection; gpt delta transgenic mouse; mitomycin C; N-propyl-N-nitrosourea; packaging efficiency (PE); Spi- (sensitive to P2 interference) selection; total population