



Title of Research:

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Development of a short-term *in vivo* assay for thyroid hormone disrupting activity in maternal rats and their fetus/pups as prescreening for developmental neurotoxicity potential.

Principal Investigator:

Tomoya Yamada, DVM, PhD (Fellow, Sumitomo Chemical Company, Ltd., Environmental Health Science Laboratory)

3-1-98 Kasugadenaka, Konohanaku, Osaka 554-8558, Japan

(e-mail) yamadat8@sc.sumitomo-chem.co.jp

Collaborators:

Hiroaki Aoyama, PhD (Executive Director, Toxicology Division, The Institute of Environmental Toxicology)

4321 Uchimoriya-machi, Joso-shi, Ibaraki 303-0043, Japan

(e-mail) aoyama@iet.or.jp

Hidenori Suto, PhD (Research Associate, Sumitomo Chemical Company, Ltd., Environmental Health Science Laboratory)

3-1-98 Kasugadenaka, Konohanaku, Osaka 554-8558, Japan

(e-mail) sutoh@sc.sumitomo-chem.co.jp

Summary of Research:

As thyroid hormones (TH) are essential for fetal brain development, the chemicals which induce reduction of serum TH levels may potentially interfere with the developing brain. Since standardized studies to identify developmental neurotoxicity (DNT) require significant resources, a simple screening test is eagerly awaited for sorting chemicals to be examined in the DNT studies. Recently, we began verifying the feasibility of a modified Comparative Thyroid Assay (CTA) by downsizing the number of rats but with the addition of examination of the brain TH levels and brain histology. The 1st study showed that the modified CTA could detect 6-propylthiouracil (6-PTU, 10 ppm)-induced severe (>70%) reduction of serum TH in dams, with >50% suppressed serum/brain TH levels and brain abnormality (heterotopia) in offspring. The modified CTA also detected sodium phenobarbital (NaPB, 1000 ppm)-induced mild (<35%) reduction of serum TH levels in dams, with mild (<35%) reduction of serum/brain TH levels in fetuses but not in pups, and without increased brain heterotopia. The 2nd study showed that the findings by NaPB at 1000 ppm were generally reproducible in dams and offspring. To investigate what degree of serum/brain TH disruptions will be adverse to brain morphology, we performed a 3rd study focusing on heterotopia formation by using treatment with a wider range of doses of 6-PTU (0, 1, 3, and 10 ppm in feed) during the critical time window for heterotopia formation (GD19-LD2). 6-PTU dose-dependently reduced serum TH in dams and serum/brain TH in offspring, and induced heterotopia formation in PND21 pups. Although detailed analysis is still in progress, our findings suggest that the modified CTA can be a potential short-term *in vivo* assay for sorting offspring TH disruptors.

Timeline:

March 1, 2022 – February 28, 2023.

Topics:

Oral presentation at JCIA LRI Annual Workshop 2021 “Development of a short-term *in vivo* assay for thyroid hormone disrupting activity in maternal rats and their fetuses/pups as prescreening for potential of developmental neurotoxicity.” (On-line, August 26th, 2022)

Publications:

Kenta Minami¹, Hidenori Suto¹, Akira Sato², Keiko Ogata¹, Tadashi Kosaka², Hitoshi Hojo², Naofumi Takahashi², Naruto Tomiyama², Takako Fukuda¹, Katsumasa Iwashita¹, Hiroaki Aoyama² and Tomoya Yamada¹ (¹ Sumitomo Chemical Company, Ltd. ² The Institute of Environmental Toxicology), 2023. Feasibility study for a downsized comparative thyroid assay with measurement of brain thyroid hormones and histopathology in rats: case study with 6-propylthiouracil and sodium phenobarbital at high dose. Regulatory Toxicology and

Kenta Minami¹, Akira Sato², Naruto Tomiyama², Keiko Ogata¹, Tadashi Kosaka², Hitoshi Hojo², Naofumi Takahashi², Hidenori Suto¹, Hiroaki Aoyama² and Tomoya Yamada¹ (1 Sumitomo Chemical Company, Ltd. 2 The Institute of Environmental Toxicology), 2023. Feasibility study for a downsized comparative thyroid assay with measurement of brain thyroid hormones and histopathology in rats: Part II. Within laboratory reproducibility for effects of sodium phenobarbital (submitted, under review)

Kenta Minami¹, Hidenori Suto¹, Akira Sato², Keiko Ogata¹, Kenta Minami¹, Tadashi Kosaka², Hitoshi Hojo², Naofumi Takahashi², Naruto Tomiyama², Hiroaki Aoyama², Tomoya Yamada¹ (1 Sumitomo Chemical Company, Ltd. 2 The Institute of Environmental Toxicology)

“Feasibility and reliability of a downsized comparative thyroid assay for evaluating thyroid hormone disrupting activity in maternal rats and their offspring: reproducibility study with sodium phenobarbital”. The 49th Annual Meeting of the Japanese Society of Toxicology, Sapporo, Japan, July 2022.

Tomoya Yamada (Sumitomo Chemical Company, Ltd.)

“Comparative Thyroid Assay: Current situation of a short-term in vivo assay for thyroid hormone disrupting activity in maternal rats and their offspring as prescreening for potential developmental neurotoxicity”. The 49th Annual Meeting of the Japanese Society of Toxicology, Sapporo, Japan, July 2022.

Keiko Ogata¹, Kenta Minami¹, Hidenori Suto¹, Hiroyuki Asano¹, Masahiko Kushida¹, Keiko Maeda¹, Akira Sato², Naofumi Takahashi², Hiroaki Aoyama², Tomoya Yamada¹ (1 Sumitomo Chemical Company, Ltd. 2 The Institute of Environmental Toxicology)

“A method evaluating brain morphology in a screening study of low thyroid hormone-related developmental neurotoxicity”. The 39th Annual Meeting of the Japanese Society of Toxicologic pathology, Tokyo, Japan, January 2023.

Tomoya Yamada¹ and Hiroaki Aoyama², (1 Sumitomo Chemical Company, Ltd. 2 The Institute of Environmental Toxicology)

“A proposal for the use of a modified comparative thyroid assay with reduced number of animals and additional parameters”. The 62nd Annual Meeting of the Society of Toxicology, Nashville, United States, To be presented on March 2023.

Kenta Minami¹, Hidenori Suto¹, Akira Sato², Keiko Ogata¹, Tadashi Kosaka², Hitoshi Hojo², Naofumi Takahashi², Naruto Tomiyama², Hiroaki Aoyama², Tomoya Yamada¹ (1 Sumitomo Chemical Company, Ltd. 2 The Institute of Environmental Toxicology)

“Effects of sodium phenobarbital in a downsized comparative thyroid assay with additional examination of brain thyroid hormone levels and brain histology”. The 62nd Annual Meeting of the Society of Toxicology, Nashville, United States, To be presented on March 2023.