# Determination of Thyroidal EDCs Activities Using Approved Human Cell-Based Transactivation Assay

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## Abstract

Endocrine-disrupting chemicals (EDCs) interfere with physiological function by mimicking or blocking hormones; these chemicals enter the human body through various materials used in food packaging, among other routes. Thyroid hormones (THs) are very important hormones that control various basic physiological functions. In a previous study, we developed a TH agonist transactivation (TA) assay based on the A549 cell line. However, the assay using A549 showed some limitations since it required 4 days to yield results and showed low sensitivity to the natural form of human triiodothyronine (T3). Therefore, in this study, we have developed a more sensitive TH TA assay based on a HeLa cell line to screen potential TH agonists. We evaluated the TH agonist activity of 17 chemicals, 5 of which showed TH agonist activity. In conclusion, in comparison with the previously developed TA assay, the assay using HeLa cells provided greater accuracy, sensitivity, and specificity, yielding more detailed results for TH agonist chemicals in less time.


## 1. Introduction

Endocrine-disrupting chemicals (EDCs) are exogenous chemical substances that disrupt body function, e.g., by imitating or blocking hormones. EDCs may disrupt hormone systems through broader mechanisms, although they exert their actions primarily through various nuclear hormone receptors such as estrogen receptors (ERs), androgen receptors (ARs), progesterone receptors, thyroid hormone receptors (THRs), and retinoid receptors [1].

Exposure to EDCs is complex; exposure can be from multiple sources and to several types of EDCs at the same time. Since most EDCs are lipophilic, bioaccumulation in higher organisms occurs through the food chain. They are generally not decomposed naturally, nor are they generally metabolized or decomposed into more toxic compounds than the original chemicals [1]. Most importantly, these chemicals are an intrinsic part of our lives, and humans are exposed to EDCs via food packaging materials, including not only plastics but also textiles, paper, wood, metal, glass, regenerated cellulose, ceramics, waxes, and cork (Diamanti-Kandarakis et al., 2009; Muncke, 2009). Experimental studies have shown that exposure to EDCs such as polychlorinated biphenyls (PCBs), dioxins, polybrominated flame retardants, pesticides, perfluorinated chemicals (PFCs), phthalates, bisphenol A (BPA), UV-filters, and perchlorate can disrupt thyroid function. While PCBs have been shown to have adverse effects on thyroid function, studies on the other chemicals have only presented the possibility of thyroid-disrupting properties [3].

Thyroid hormones (THs) play an important role in cell proliferation and differentiation [4], growth and development [56], and homeostasis [7], as well as various physiological functions such as regulation of metabolism [89]. To identify potential thyroidal EDCs, it is necessary to establish test methods that can provide information regarding the potential of chemicals to interfere with aspects of the thyroid pathway, including interaction with the THR and transactivation (TA) of genes via the binding to the THR. We had previously developed an in vitro method for screening chemicals that interact with the based on human A549 cells [10]. However, this TH agonist transactivation (TA) assay using A549 cells required 4 days to yield results and showed a low sensitivity for endogenous T3. Therefore, we developed an agonist TH TA assay based on the human cervix adenocarcinoma HeLa cell line that showed a higher THR expression level and yielded enhanced performance.

## 2. Results And Discussion

# 2.1. Preparation of a stably transfected HeLa cell line.

In the previous study, we constructed a TH-responsive dual-reporter cassette using retrovirus to establish a stably transfected cell line. The TH-responsive dual-reporter cassette consisted of a TRE region, a minimal 15-bp TATA box, secNluc luciferase, and IRES-EGFP. Correct construction of the TH-responsive dual-reporter cassette was confirmed by DNA sequencing [10]. TRs are expressed in several human organ tissues, including brain, breast, cervix, kidney, liver, lung, pancreas, and stomach tissues [10]. To develop a human cell-based TH agonist TA assay, we used TH-responsive dual reporter assay

system based on previously established A549 system to transfect HeLa cells. This human cell-based assay used an innate transcription system controlled by TH. Therefore, we selected and used HeLa cells, a human cervix adenocarcinoma cell line with a higher TR expression level than the previously used A459 cell line. To establish stably transfected HeLa cells, we used the TH-responsive dual-reporter cassette, since stably transfected A549 cells showed stable T3-mediated EGFP and secNluc luciferase gene expression in the previous study.

## 2.2. Confirmation of T3-mediated EGFP and secNluc luciferase gene expression in stabilized HeLa cells with the transfected dual-reporter system.

HeLa-TRE-Nluc-EGFP cells were treated for 24 h with (3,3',5-triiodo-L-thyronine, +T3) or without (-T3) 100 nM T3. Nucleus: Hoechst 33342 (blue); EGFP: expressed in Hela-TRE-Nluc-EGFP cells (green).

A retrovirus harboring the TRE-secNLuc-IRES-EGFP reporter cassette was used to transfect the TH-responsive dual-reporter system into the HeLa cell line. We treated each clone with 100 nM T3 and evaluated T3-mediated EGFP expression to select HeLa cells transfected with TH-responsive dual-reporter system. The T3-mediated EGFP expression in HeLa cells was induced by treatment with 100 nM T3. These results established that the TH-responsive dual-reporter system in HeLa cells was controlled by T3 (Fig. 1).

The HeLa cells stably transfected were exposed to T3 (10 pM to 10 μM). The mean induction-fold in comparison with the vehicle control (VC) is presented (n = 3).

To evaluate TH agonistic properties of the test chemicals, we measured the T3-mediated secNluc luciferase gene expression. The HeLa cells were treated with T3 in a dose-dependent manner at seven concentrations (each concentration diluted 10-fold, from $10^{-5}$ ~ to $10^{-11}$ M) to evaluate the effect of T3 on TRE-induced secNluc luciferase gene expression. As the concentration of T3 increased, secNluc luciferase activity increased in a dose-dependent manner compared to the activity of vehicle control. The increase ratio of secNluc luciferase activity with 100 nM T3 was consistently 6-fold or higher compared to the vehicle control (Fig. 2). The mean value of log[$EC_{10}$(M)] was − 9.84 and that of log[$EC_{50}$(M)] was − 8.71. In the “Rat pituitary tumor cell (GH3) reporter gene assay” in OECD GD 207 ‘Ranking Parameter Analysis’ of TR transactivation, the log[$EC_{10}$(M)] and log[$EC_{50}$(M)] values induced by T3 were − 11 and − 10, respectively [11]. Thus, this stabilized HeLa cell line transfected with a thyroid-responsive dual-reporter system showed sensitivity to T3 similar to that of the rat pituitary cell line. The stably transfected A549 cells required incubation for 72 h after T3 treatment until sufficient amounts of luciferase were secreted in the supernatant [10]. In contrast, the stably transfected HeLa cells required incubation for only 48 h after T3 treatment until sufficient amounts of luciferase were secreted in the supernatant. Thus, the TH agonist TA assay using stably transfected HeLa cells required less time for measurement of luminescence than the assay using A549 cells. This advantage suggests that the assay with HeLa cells offers more speed and sensitivity than the assay with A549 cells.

## 2.3. Transactivation assay with potential TH agonistic chemicals.

The secNluc luminescence units induced by the 17 chemicals are represented as the % activity observed with reference to the activity of 100 nM T3 (n = 3).

To evaluate the developed TA assay, 17 chemicals were selected for testing their potential TH agonistic activity based on the OECD GD 207 [12]. Five of these 17 chemicals (T3, T4, Triac, Tetrac, GC-1) showed TH agonist activity based on the log[$EC_{10}$(M)] and log[$EC_{50}$(M)] values and showed the same results as GD 207 (Fig. 3). The mean values of log[$EC_{10}$(M)] and log[$EC_{50}$(M)], the standard deviation of triplication, and the coefficient of variation (%CV) are summarized in Table 1.

Table 1
Log[$EC_{10}$(M)] and Log[$EC_{50}$(M)] values from the TH agonist TA assay

| No. | Test chemicals | Log[$EC_{10}$(M)] | | | | | | Log[$EC_{50}$(M)] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | #1 | #2 | #3 | Avg. | SD | %CV | #1 | #2 | #3 | Ave | SD | %CV |
| 1 | T3 | -9.89 | -9.72 | -9.69 | -9.76 | 0.11 | 1.12 | -8.73 | -8.45 | -8.45 | -8.54 | 0.16 | 1.87 |
| 2 | T4 | -7.83 | -7.77 | -7.75 | -7.78 | 0.04 | 0.55 | -6.96 | -6.75 | -6.74 | -6.82 | 0.12 | 1.77 |
| 3 | Glycitein | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | BP-2 | - | - | - | - | - | - | - | - | - | - | - | - |
| 5 | E2 | - | - | - | - | - | - | - | - | - | - | - | - |
| 6 | BPA | - | - | - | - | - | - | - | - | - | - | - | - |
| 7 | Tetrac | -7.62 | -7.63 | -7.60 | -7.61 | 0.02 | 0.23 | -6.18 | -6.29 | -6.24 | -6.24 | 0.05 | 0.86 |
| 8 | GC-1 | -8.91 | -8.94 | -8.99 | -8.95 | 0.04 | 0.48 | -6.87 | -6.94 | -7.46 | -7.09 | 0.32 | 4.52 |
| 9 | Triac | -9.68 | -9.78 | -9.86 | -9.77 | 0.09 | 0.94 | -8.14 | -8.27 | -8.46 | -8.29 | 0.16 | 1.91 |
| 10 | Amiodarone hydrochloride | - | - | - | - | - | - | - | - | - | - | - | - |
| 11 | Hispidulin | - | - | - | - | - | - | - | - | - | - | - | - |
| 12 | Procymidone | - | - | - | - | - | - | - | - | - | - | - | - |
| 13 | Bithionol | - | - | - | - | - | - | - | - | - | - | - | - |
| 14 | Closantel | - | - | - | - | - | - | - | - | - | - | - | - |
| 15 | Rafoxanide | - | - | - | - | - | - | - | - | - | - | - | - |
| 16 | 4,4'-Diiodobiphenyl | - | - | - | - | - | - | - | - | - | - | - | - |
| 17 | BDE No 28 | - | - | - | - | - | - | - | - | - | - | - | - |

T3: 3,3',5-Triiodo-l-thyronine sodium salt; T4: Levothyroxine sodium; BP-2: 2,2',4,4'-Tetrahydroxybenzophenone; E2: 17β-Estradiol; BPA: Bisphenol A; Tetrac: 3,5,3',5'-Tetraiodo Thyroacetic Acid; GC-1: Sobetirome; Triac: 3,3',5-Triiodothyroacetic acid

T3 and T4 are the major forms of human TH. Although T4 is secreted in relatively higher amounts than T3 in the human body, the TH agonist TA activity of T3 was higher than that of T4 because the binding affinity of T3 to TR is 10-fold higher than that of T4 and because T3 primarily plays the role of a transcription ligand [13]. Other chemicals that showed TH agonist TA-positive activity (Triac, Tetrac, and GC-1) show structural similarities as natural TH derivatives. Thus, Triac, Tetrac, and GC-1 showed relatively weak TH agonist TA activity. We also measured %CV for triplicates was measured to confirm the accuracy and repeatability of the assessments. Very low %CV values were obtained, suggesting good reproducibility of the TH agonist TA assay using stably transfected HeLa cells.

Table 2
Log[$EC_{10}$(M)] and Log[$EC_{50}$(M)] values from the TH agonist TA assay (subdivided concentrations)

| No. | Test chemicals | Log[$EC_{10}$(M)] | | | | | | Log[$EC_{50}$(M)] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | #1 | #2 | #3 | Ave | SD | %CV | #1 | #2 | #3 | Ave | SD | %CV |
| 1 | T3 | -9.31 | -9.25 | -9.39 | -9.32 | 0.07 | 0.75 | -8.11 | -7.86 | -8.01 | -7.99 | 0.13 | 1.58 |
| 2 | T4 | -8.45 | -8.77 | -8.33 | -8.52 | 0.22 | 2.64 | -7.73 | -7.94 | -7.59 | -7.75 | 0.18 | 2.28 |
| 3 | Tetrac | -7.55 | -7.61 | -7.51 | -7.56 | 0.05 | 0.71 | -6.53 | -6.68 | -6.57 | -6.59 | 0.08 | 1.15 |
| 4 | GC-1 | -9.16 | -9.11 | -9.03 | -9.10 | 0.07 | 0.73 | -8.20 | -7.99 | -7.92 | -8.04 | 0.14 | 1.80 |
| 5 | Triac | -9.36 | -9.24 | -9.33 | -9.31 | 0.07 | 0.70 | -8.48 | -8.52 | -8.62 | -8.54 | 0.07 | 0.83 |

T3: 3,3',5-Triiodo-l-thyronine sodium salt; T4: Levothyroxine sodium; BPA: Bisphenol A; Tetrac: 3,5,3',5'-Tetraiodo Thyroacetic Acid; GC-1: Sobetirome; Triac: 3,3',5-Triiodothyroacetic acid

Furthermore, the 5 positive chemicals were further tested with additional concentrations to provide more detailed data (Fig. 4). The mean values of log[$EC_{10}$(M)] and log[$EC_{50}$(M)], the standard deviation, and %CV of triplication are summarized in Table 2. Similar to the tests for the 17 chemicals, the %CV values of 5 selected chemicals were very low values. These results indicate that the TH agonist TA assay using stably transfected HeLa cells developed in this study showed very high reproducibility for screening potential TH agonistic chemicals. Furthermore, in a comparison of the dose-dependent responses of each positive chemical in the TH agonist TA assays using A549 and HeLa cells, the TH agonist TA assay using HeLa showed higher induction levels than the assay using A549 cells at all concentration, indicating the thyroid TA activity of each chemical. Higher level of induction indicates that the stably transfected HeLa cells responded more sensitively to chemicals mimicking thyroid hormones. These results indicated that the TH TA assay using HeLa could provide more sensitive thyroid TA activity information of test chemicals than the TH agonist TA assay using A549 cells. Moreover, the TH agonist TA assay using A549 cells required 4 days to yield results, which is a relatively long time for high-throughput screening. Therefore, we developed the TH TA assay using HeLa cells, which redressed some of the limitations of the test method using A549. The TH agonist TA assay using HeLa cells could provide accuracy, sensitivity, and specificity.

With the increasing risks associated with EDCs, the development of appropriate test methods for management and assessment of these EDCs has become important. This is especially pertinent because the thyroid hormone is involved in energy metabolism and homeostasis, necessitating the development of practical test methods to assess EDCs that disrupt thyroid hormones. The TH TA assay in this study was suitable for assessment and analysis of EDCs that mimic TH in the human body because of the use of the using human HeLa cell line.

## 3. Materials And Methods

## 3.1. Chemicals.

The reference chemicals 3,3',5-triiodo-L-thyronine salt (T3, CASRN 55-06-1) and 7,4'-dihydroxy-6-methoxyisoflavone (glycitein, CASRN 40957-83-3) were purchased from Sigma-Aldrich (St. Louis, USA), and 3,3',5,5'-tetraiodo-L-thyronine (T4, CASRN 55-03-8) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Other test chemicals were purchased from various commercial sources including Sigma-Aldrich (St. Louis, USA), Tokyo Chemical Industry (Tokyo, Japan), Toronto Research Chemicals (North York, Canada), and Santa Cruz Biotechnology (Dallas, USA). Table 3 presents a list of the chemicals used for the TH agonist TA assay.

Table 3
Chemicals used for the TH agonist TA assay

| No. | Chemical | Supplier | CASRN | Cat. No. | Test range [Log (M)] |
|---|---|---|---|---|---|
| 1 | 2,2',4,4'-Tetrahydroxybenzophenone (BP-2) | Sigma | 131-55-5 | T16403-25G | -10.00 to -4.00 |
| 2 | Levothyroxine sodium (T4) | TCI | 55-03-8 | T0245 | -11.00 to -5.00 |
| 3 | 17β-Estradiol (E2) | Sigma | 50-28-2 | E1024-1G | -12.00 to -6.00 |
| 4 | Bisphenol A (BPA) | Sigma | 80-05-07 | 42088-100MG | -10.00 to -4.00 |
| 5 | 3,3',5-Triiodo-l-thyronine sodium salt (T3) | Sigma | 55-06-1 | T6397-100MG | -11.00 to -5.00 |
| 6 | 3,5,3',5'-Tetraiodo Thyroacetic Acid (Tetrac) | TRC | 67-30-1 | T296125 | -11.00 to -5.00 |
| 7 | Glycitein | Sigma | 40957-83-3 | 43534-10MG | -11.00 to -5.00 |
| 8 | Sobetirome (GC-1) | Sigma | 211110-63-3 | SML1900-25MG | -10.00 to -4.00 |
| 9 | 3,3',5-Triiodothyroacetic acid (Triac) | Sigma | 51-24-1 | T7650-100MG | -11.00 to -5.00 |
| 10 | Amiodarone hydrochloride | Sigma | 19774-82-4 | A8423-1G | -12.00 to -6.00 |
| 11 | Hispidulin | SANTA CRUZ | 1447-88-7 | sc-203999 | -11.00 to -5.00 |
| 12 | Procymidone | Sigma | 32809-16-8 | 36640 | -11.00 to -5.00 |
| 13 | Bithionol | Sigma | 97-18-7 | SML1440 | -11.00 to -5.00 |
| 14 | Closantel | Sigma | 57808-65-8 | 34093 | -11.00 to -5.00 |
| 15 | Rafoxanide | Sigma | 22662-39-1 | 34042 | -11.00 to -5.00 |
| 16 | 4,4'-Diiodobiphenyl | Sigma | 3001-15-8 | D122408 | -12.00 to -6.00 |
| 17 | BDE No 28 | Sigma | 41318-75-6 | 5364 | -11.00 to -5.00 |

Sigma: Sigma-Aldrich Co, LLC; TCI: Tokyo Chemical Industry Co, LTD; TRC: Toronto Research Chemicals, Inc SANTA CRUZ: Santa Cruz Biotechnology, Inc.

## 3.2. Cell culture.

HeLa cells (ATCC, Manassas, USA), the human cervix adenocarcinoma cell line, were maintained in growth medium consisting of minimum essential medium (MEM; Gibco, Grand Island, USA) containing 10% fetal bovine serum (FBS; Gibco, Grand Island, USA), and 100 U/mL penicillin-100 µg/mL streptomycin (Pen-Strep; Gibco, Grand Island, USA) and incubated in a 5% $CO_2$ atmosphere at 37˚C. After transfection, the transfected HeLa cells were maintained in selection medium consisting of MEM containing 10% FBS, 1 × Pen-Strep, and 200 µg/mL Hygromycin B Gold (Invivogen, San Diego, USA).

## 3.3. Retrovirus harboring the TRE-secNluc-IRES-EGFP reporter cassette.

A TH-responsive dual-reporter assay system was constructed using the method described previously (Lee et al., 2020). To insert the TRE-secNluc-IRES-EGFP cassette into the pLPCX vector (Clontech, Mountain View, USA), the BglII-ClaI-digested

PCR fragment containing the TRE-secNluc-IRES-EGFP cassette was inserted into the BglII-ClaI sites of pLPCX. The structure of the TRE-secNluc-IRES-EGFP/pLPCX was verified by DNA sequencing [10].

The 293GPG packaging cell line was cultured according to the method specified elsewhere [10]. Cells were plated at $1.0 \times 10^5$ cells/well in a 60-mm culture dish to produce the TRE-secNluc-IRES-EGFP-harboring retrovirus. When the cell confluence reached 80%, the cells were transfected with 0.8 µg of the TRE-secNluc-IRES-EGFP/pLPCX reporter by using Lipofectamine® 3000 transfection reagent (Invitrogen, Carlsbad, USA) for 18hrs. The medium was replaced with fresh 293GPG medium excluding tetracycline, puromycin, or G418, and the viral supernatant was then collected up to 72 h every 24 h. The supernatants were filtered using filters with a pore size of 0.45 µm (Thermo Fisher Scientific, Waltham, USA) and stored at -80°C.

## 3.4. Stably transfected HeLa cell line.

HeLa cells were plated at $5.0 \times 10^4$ cells/well in a 12-well culture plate with growth medium and incubated at 24 h. The next step involved treating the HeLa cells with 500 µL per well of stored retrovirus for 18 h to allow transfection of the TRE-secNluc-IRES-EGFP construct. After 18 h, the growth medium was replaced with fresh growth medium without hygromycin B Gold for stabilization of the transfected HeLa cells and incubated for 6 h. The transfected HeLa cells were transferred to a 100-mm culture dish for pre-selection and incubated for 24 h.

The growth medium was removed, and the cells were maintained with the selection medium, which is consisted of the growth medium with 200 µg/mL hygromycin B Gold, for 4 days. During the four days of incubation, the selection medium was replaced with the fresh selection medium on the 48 h, the cells were split at 1/30 ratio with fresh selection medium on day four to separate into single cells. When the separated cells were maintained for 48 h, the single colonies harvested using a cloning cylinder (Corning, Glendale, USA) and transferred to a 12-well plate, respectively. The single colonies were incubated in a 5% CO2 atmosphere at 37℃ for 11 days. The selection medium was replaced by fresh selection medium every 2 days. The isolated cells in the 12-well plate were split at a 1/3 ratio in three 12-well plates to confirm T3 (100 nM)-mediated EGFP expression and for maintenance. Among the clones derived from the 12 single colonies secured, one clone selected, from the 12 single-colonies, for the use in the TA assay based on EGFP expression which is mediated by T3.

## 3.5. Transactivation assay.

The stable cell line was maintained in a culture medium containing 200 µg/mL hygromycin B Gold. For the assay, stably transfected HeLa cells were seeded into 96-well white cell culture microplates (Corning, Glendale, USA) ($5.0 \times 10^3$ cells/well). The assay medium was MEM without phenol red and L-glutamine (Gibco, Grand Island, USA) including 10% dextran-coated charcoal-treated FBS (DCC-FBS) with 100 U/mL penicillin-100 µg/mL streptomycin, 2 mM L-glutamine (Gibco, Grand Island, USA), and 200 µg/mL hygromycin B, and incubated in a 5% CO2 atmosphere at 37℃ for 24 h. The cells were treated with the test chemicals (Table 3) by replacing the assay medium containing intended concentration of chemicals.

A 7-point 10-fold serial dilution of the chemical was triplicated for each concentration and evaluated in the TA assay. The test chemicals were diluted with the assay medium containing 10 pM 9-cis-retinoic acid (9cRA). Vehicle and positive control (VC and PC) samples were obtained by adding 0.1% DMSO to assay medium containing 10 pM 9cRA and 100 nM T3 to assay medium containing 10 pM 9cRA, respectively. After 48 h of chemical treatment, Luciferase activities were measured by using the Nano-Glo® Luciferase Assay System (Promega, Madison, USA).

## 3.6. Data acquisition and statistical analyses.

The luminescence was determined at 495–505 nm using a GloMax® Discover Microplate Reader (Promega, Madison, USA). The average value for the positive control (PC, 100 nM T3) was calculated as the ratio to the vehicle control (VC, 0.1% DMSO).

The agonistic activity was calculated from RLU units as follows:

$$\text{Agonistic activity (\%)} = \frac{\text{Mean RLU of test chemical - Mean RLU of VC}}{\text{Mean RLU of PC - Mean RLU of VC}} \times 100$$

The induction-fold corresponding to the $PC_{10}$ value (the concentration of chemical estimated to cause 10% activity of the positive control response) should be greater than 1 ± 2 standard deviations (SDs) of the induction-fold value (= 1) for the corresponding VC [14].

# 4. Conclusion

We developed a TH TA assay based upon HeLa cells with a TRE-secNluc-IRES-EGFP reporter. The TH TA assay using HeLa cells showed improved performance for screening of potential TH agonistic chemicals Compared to the TH agonist TA assay using stably transfected A549 cells from the previous study. The TH TA assay using stably transfected HeLa cells developed in this study provided more sensitive results in comprehensive testing, yielded faster results. Therefore, the developed TH agonist TA assay using HeLa cells can be used as a plausible method detecting thyroid agonistic chemicals.

# Abbreviations

EGFP, enhanced green fluorescent protein; GD, Guidance Document; RCB, polychlorinated biphenyl; PFC, perfluorinated chemical; BPA, bisphenol A; T3, 3,3',5-triiodo-L-thyronine salt; glycitein, 7,4'-dihydroxy-6-methoxyisoflavone; IRES, internal ribosome entry site; T3, triiodothyronine; T4, 3,3',5,5'-tetraiodo-L-thyronine; TRE, T3 response element; sNluc, secreted Nanoluc luciferase;

# Declarations


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### Authors' contribution

H.S. and S-H.L. wrote the main manuscript text and prepared figure 1-4. H-S.L. validated data and reviewed the manuscript. Y.P. administered this project, acquired funding and reviewed the manuscript. All authors, including C.W. and N.B. reviewed the manuscript.

### Competing interests

The authors declare no competing interests.

# References


1. Diamanti-Kandarakis, E. *et al.* Endocrine-disrupting chemicals: An Endocrine Society scientific statement. *Endocr. Rev*, **30**, 293–342 (2009).
2. Muncke, J. Exposure to endocrine disrupting compounds via the food chain: Is packaging a relevant source? *Sci. Total Environ*, **407**, 4549–4559 (2009).
3. Boas, M., Feldt-Rasmussen, U. & Main, K. M. Thyroid effects of endocrine disrupting chemicals. *Mol. Cell. Endocrinol*, **355**, 240–248 (2012).
4. Pascual, A. & Aranda, A. Thyroid hormone receptors, cell growth and differentiation. *Biochim. Biophys. Acta - Gen. Subj*, **1830**, 3908–3916 (2013).

5. Bernal, J. Thyroid hormone receptors in brain development and function. *Nature Clinical Practice Endocrinology and Metabolism*, https://doi.org/10.1038/ncpendmet0424 (2007).
6. Horn, S. & Heuer, H. Thyroid hormone action during brain development: More questions than answers. *Mol. Cell. Endocrinol*, **315**, 19–26 (2010).
7. Warner, A. & Mittag, J. Thyroid hormone and the central control of homeostasis. *J. Mol. Endocrinol*, **49**, 29–35 (2012).
8. Cheng, S. Y., Leonard, J. L. & Davis, P. J. Molecular aspects of thyroid hormone actions. *Endocr. Rev*, **31**, 139–170 (2010).
9. Yen, P. M. Physiological and molecular basis of Thyroid hormone action. *Physiol. Rev*, **81**, 1097–1142 (2001).
10. Lee, S. H., Seo, H., Lee, H. S. & Park, Y. Development and characterization of a human cell line-based transactivation assay to assess thyroid EDCs. *Environ. Res*, **182**, 109110 (2020).
11. Freitas, J. *et al.* Detection of thyroid hormone receptor disruptors by a novel stable in vitro reporter gene assay. *Toxicol. Vitr*, **25**, 257–266 (2011).
12. OECD. *New scoping document on in vitro and ex vivo assays for the identification of modulators of thyroid hormone signalling.Development*(2014).
13. Oetting, A. & Yen, P. M. New insights into thyroid hormone action. *Best Pract. Res. Clin. Endocrinol. Metab*, **21**, 193–208 (2007).
14. Lee, H. S., Lee, S. H. & Park, Y. Enhancement of androgen transcriptional activation assay based on genome edited glucocorticoid knock out human prostate cancer cell line. *Environ. Res*, **171**, 437–443 (2019).

## Figures

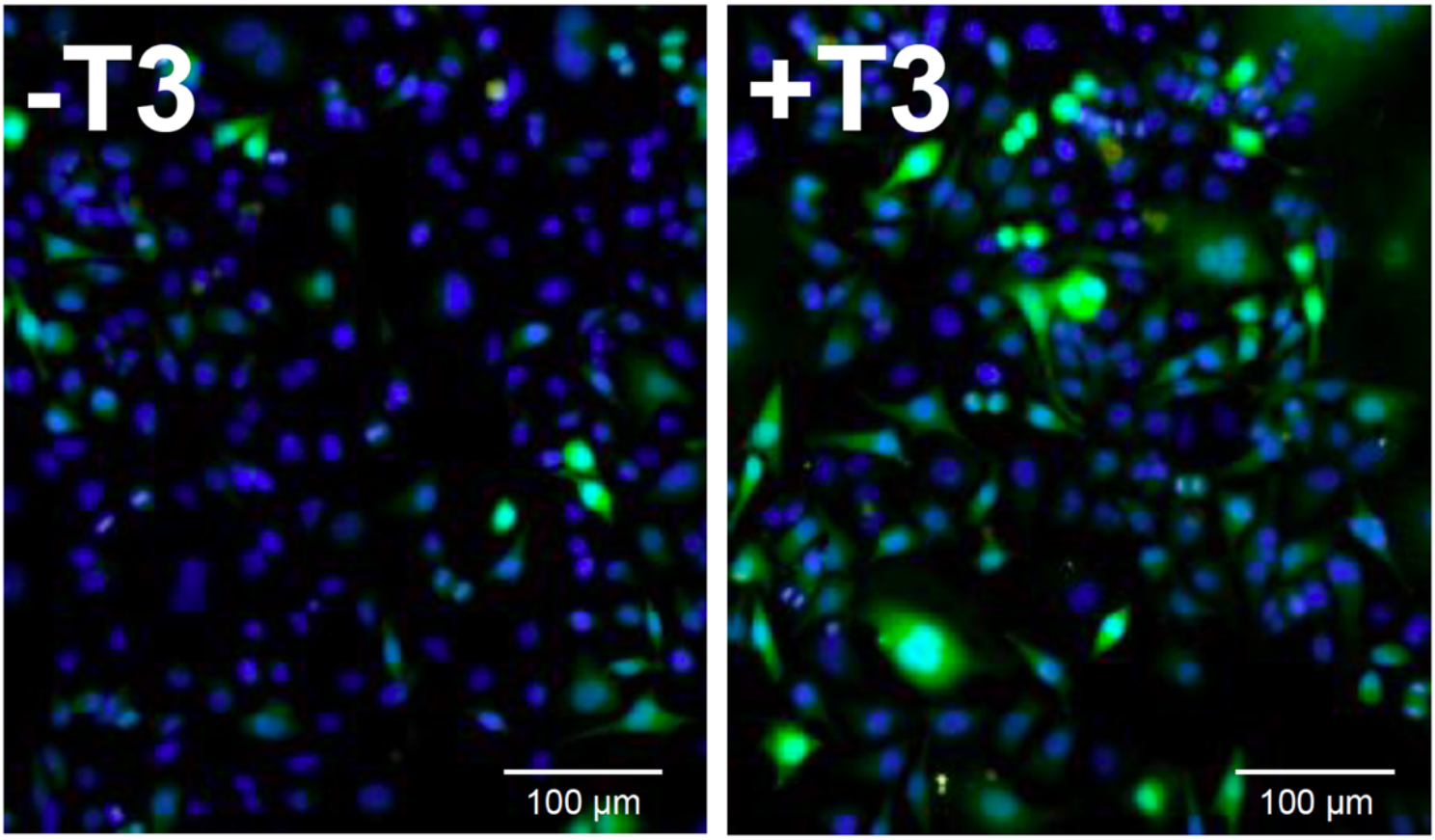



**Figure 1**

Fluorescence images of HeLa cells after T3 exposure HeLa-TRE-Nluc-EGFP cells were treated for 24 h with (3,3',5-triiodo-L-thyronine, +T3) or without (-T3) 100 nM T3. Nucleus: Hoechst 33342 (blue); EGFP: expressed in Hela-TRE-Nluc-EGFP cells (green).

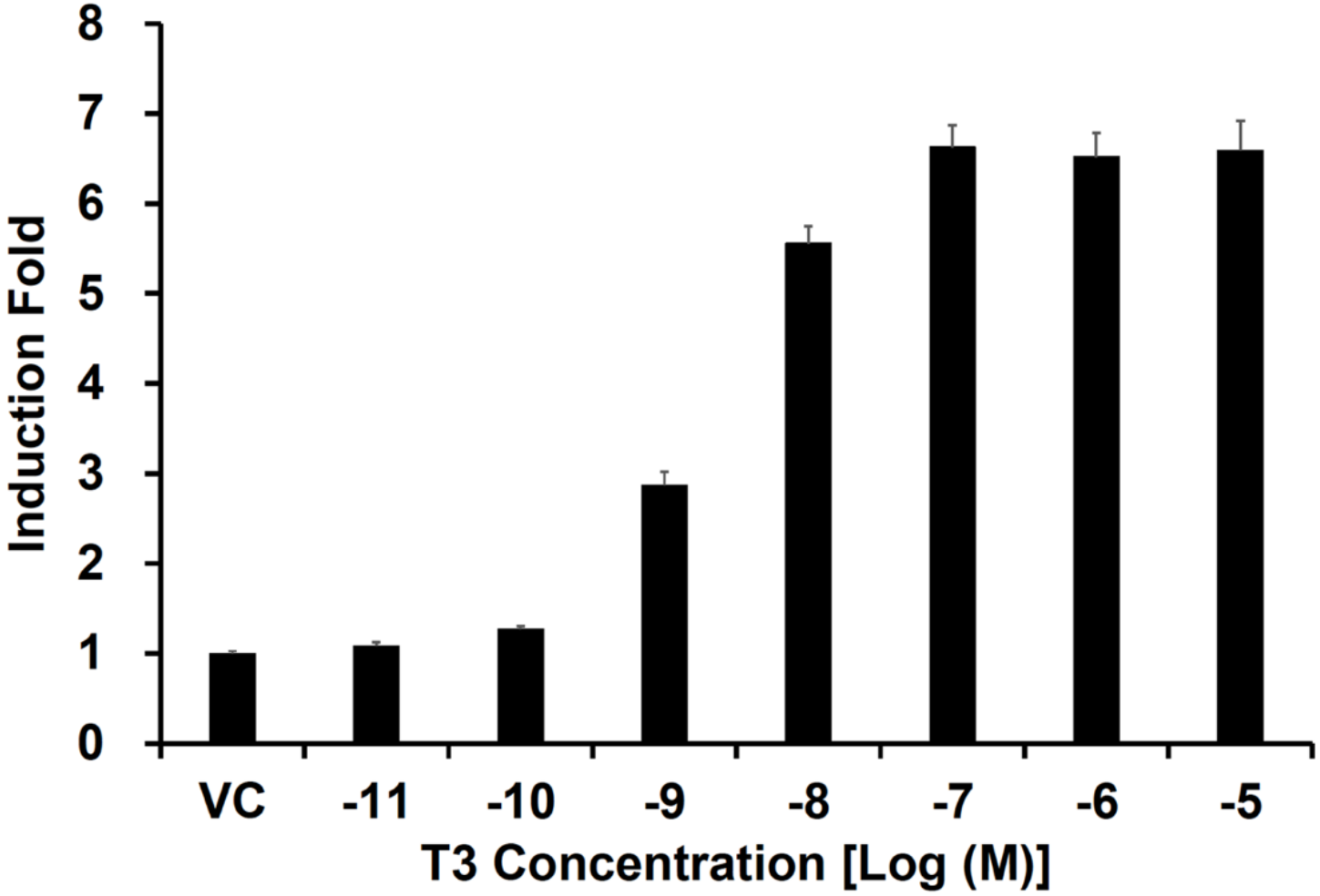
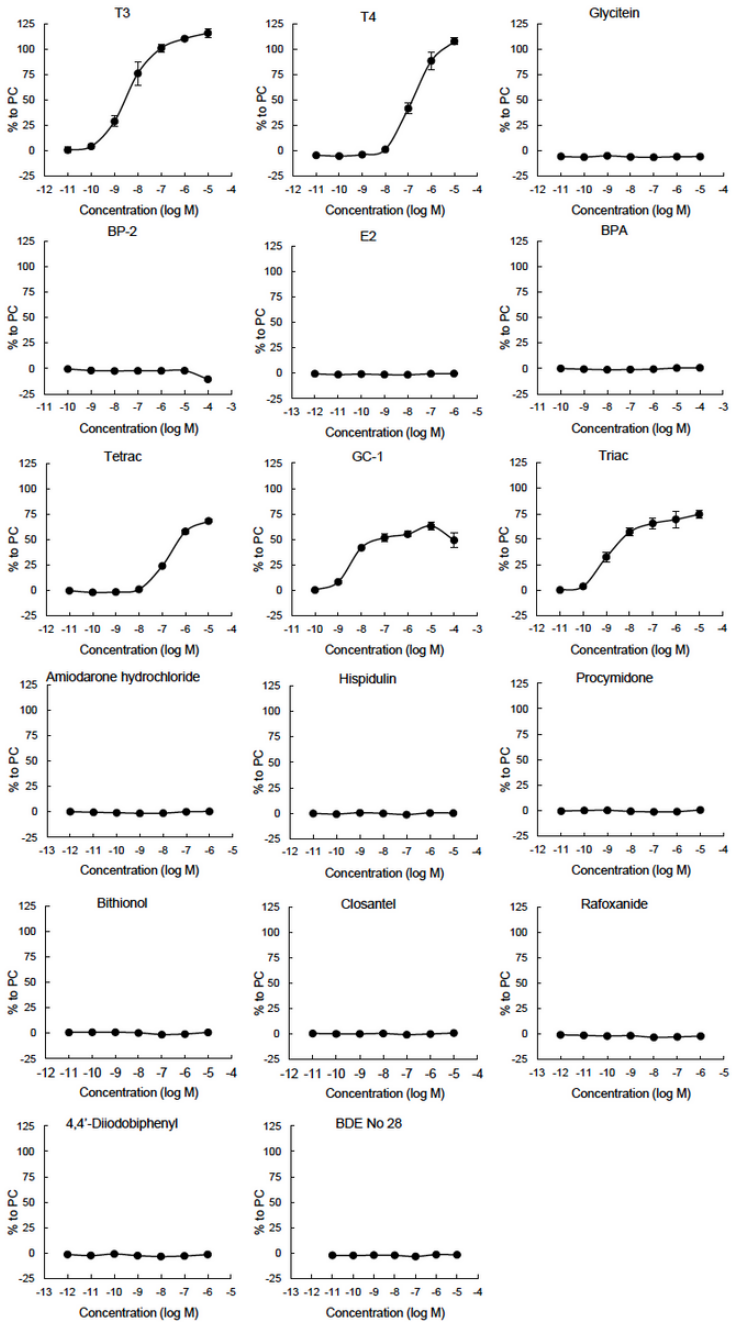



**Figure 2**

Dose-dependent response of stably transfected HeLa cell to T3 The HeLa cells stably transfected were exposed to T3 (10 pM to 10 μM). The mean induction-fold in comparison with the vehicle control (VC) is presented (n=3).




## Figure 3

Dose-dependent TH agonistic properties of the test chemicals used in the TH agonist TA assay The secNluc luminescence units induced by the 17 chemicals are represented as the % activity observed with reference to the activity of 100 nM T3 (n=3).

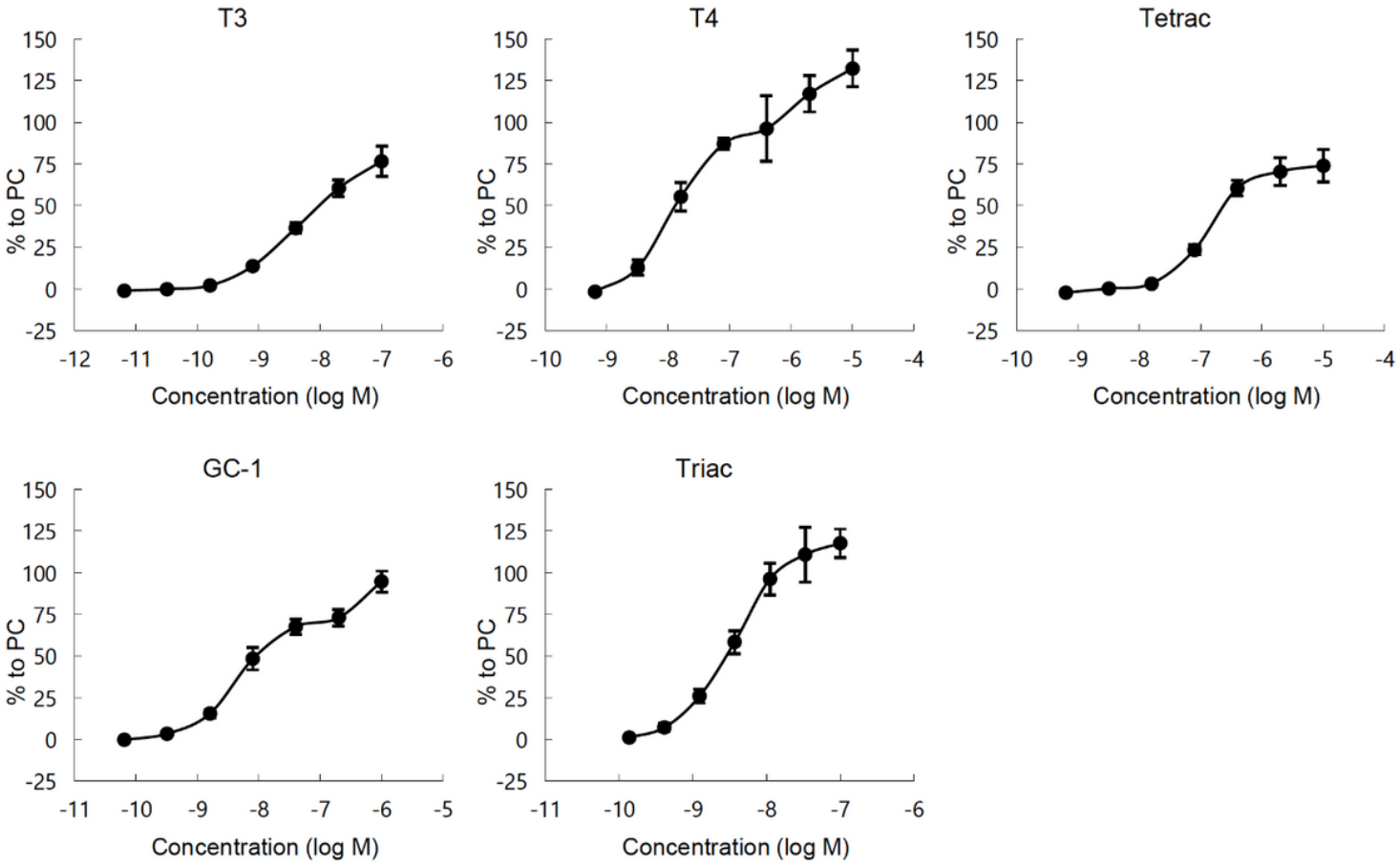



**Figure 4**

Dose-dependent TH agonistic properties of the 5 chemicals used in the TH agonist TA assay (subdivided concentration) The secNluc luminescence units induced by the 5 positive chemicals are represented as the % activity observed with reference to the activity of 100 nM T3 (n=3).

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaltable.pdf