— Regular Article —

Estrogenic Activity of Alkyl(thio)phenols and 4,4′-thiodiphenol Formed from Degradation of Commercial Insecticides

Kenta Yamada, Masanori Terasaki, and Masakazu Makino*

Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, 52–1 Yada, Suruga, Shizuoka 422–8526, Japan

(Received December 29, 2009; Accepted December 10, 2010; Published online December 15, 2010)

We investigated the estrogenic activity of commercial insecticides fenamiphos, fenthion, methiocarb, propaphos, sulprophos, and temephos as well as some phenolic compounds obtained as a result of their degradation. Using a yeast two-hybrid assay, the relative activities of 4-(methylthio)phenol, 3-methyl-4-(methylthio)phenol, 3,5-dimethyl-4-(methylthio)phenol, and 4,4′-thiodiphenol (TDP) were evaluated as 11, 10, 4, and 1000% (10 times) that of bisphenol-A. To reveal the binding abilities of the abovementioned phenolic compounds with respect to human estrogen receptor α (hERα), we carried out ER-ELISA and found that all compounds had significant abilities, particularly, TDP. From the viewpoint of bioisosterism, we discussed the similarity between a vinylene-group, –CH=CH–, and a thioether-group, –S–. We suggest that an alkylthio-group substituted at the "*para*"-position of a phenol ring plays a key role in the binding abilities of the investigated phenolic compounds.

Key words —— alkyl(thio)phenol, estrogenic activity, bioisosterism

INTRODUCTION

Organophosphorus and carbamate insecticides (OP-CMIs) are generally used for pest control in agriculture, such as in the cultivation of rice and/or fruits. Given their low cost, broad-spectrum effectiveness, and multi-pest control capability, they have been also utilized in non-agricultural applications including pet-insect, mosquito, and gnat controls.[1,2] However, at the same time, it has been pointed out that a portion of crops, and some environmental media such as surface water or soils, are contaminated with OP-CMIs.[3–5] In addition, it has been suggested that these may affect human health or ecological systems as a result of unintentional bioactivity.[6–9]

OP-CMIs exhibit inhibition of the enzyme cholinesterase, and mainly transform or degrade into two kinds of molecules known as oxon- and phenolic-compounds.[10–12] The former, derived from oxidation processes such as biological metabolism, exhibit a more marked ability to inhibit cholinesterase than do OP-CMIs themselves. The latter, induced from further metabolism or degradation through photo-irradiation, for example, show only a weak ability to inhibit cholinesterase. Therefore, in terms of inhibiting the enzyme cholinesterase, the phenolic-compounds have been regarded as a more secure product than OP-CMIs and their oxon-compounds. In agricultural chemistry, in order to maintain the inhibition (or in other words, to decrease the rate of degradation), one or more sulfur atoms have been inserted into the molecular skeleton of OP-CMIs. This is because sulfur atoms tend to be oxidized to sulfoxide and then to sulfone moieties, an outcome that is preferable to that of degradation under oxidative conditions.[13,14]

In the 1990s, it was pointed out that a portion of certain synthetic chemicals can disrupt the sensitive endocrine systems of humans or other vertebrates by mimicking or inhibiting endogenous hormones such as estrogens and/or androgens.[15] These chemicals are called endocrine disrupting chemicals (EDCs), and it has been suspected that various commercial insecticides may be EDCs, given that they are popular and ubiquitous. Thus, many

*To whom correspondence should be addressed: Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, 52–1 Yada, Suruga, Shizuoka 422–8526, Japan. Tel. & Fax: +81-54-264-5785; E-mail: makinom@u-shizuoka-ken.ac.jp

in vitro and *in vivo* bioassays have been carried out for insecticides including OP-CMIs.[16–19] While it has been determined that OP-CMIs do not cause serious and urgent effects on human health or ecological systems, it has also been pointed out that their environmental persistence and/or fate should be continuously investigated from the perspective of total risk and life cycle assessment.[20–23] Furthermore, it was found that the estrogenic potency of some phenolic-compounds, in particular, bisphenol-A (BPA), should be investigated closely since many (but not all) of them possess a common feature, displaying substantial estrogen binding activity from structure-activity relationships (SAR).[17] From SAR analysis, Nishihara *et al.* deduced that most estrogenic compounds have a phenol ring with a moiety of appropriate hydrophobicity at its "*para*"-position.[17] Here, it should be noted that the estrogenic potency of alkyl(thio)phenols such as a methyl(thio)phenol has not yet been evaluated in detail. In addition, not only can the alkylthio moiety be regarded as a kind of hydrophobic one, but the phenolic-compounds substituted with the moiety at their "*para*"-position are also the main degradation products for commercial insecticides known as fenamiphos, fenthion, methiocarb, propaphos, sulprophos, and temephos.[24–29]

In the present study, we aimed to evaluate the estrogenic potency of three alkyl(thio)phenols and 4,4′-thiodiphenol (TDP) by using the yeast two-hybrid assay incorporating the human estrogen receptor α (hERα) and hERα competitive enzyme-linked immunosorbent assay (ER-ELISA). In addition, we compared their potency with that of the insecticides themselves. Furthermore, the estimated binding ability to hERα was discussed on the basis of bioisosteric replacement between a vinylene-group, –CH=CH–, and a thioether-group, –S–, in the compounds.

MATERIALS AND METHODS

Chemicals —— 3-methyl-4-(methylthio)phenol (MMP, > 95.0% purity), 4-(methylthio)phenol (MTP, > 98.0% purity), and TDP (> 98.0% purity) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Fenamiphos (92.5% purity), fenthion (98.0% purity), methiocarb (98.5% purity), sulprofos (90.0% purity), temephos (96.0% purity) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Propaphos (> 97.0% purity) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). These chemicals were used for measurement without further purification. All other reagents used were of the purest grade and commercially available. Figure 1 shows the test compounds including 3,5-dimethyl-4-(methylthio)phenol (DMP, Fig. 1I; see bellows). Hereafter, we refer to the compounds illustrated in Fig. 1G–1J as *p*S-alkyl phenols.

Synthesis and Characterization —— DMP was synthesized by sulfenylation of 3,5-dimethylphenol using dimethyl disulfide under acidic conditions.[30] A mixture of 3,5-dimethylphenol (6.1 g, 50 mmol) and sulfuric acid (0.2 g) was heated to 170°C and then cooled 155°C. Dimethyl disulfide (4.7 g, 50 mmol) was added over a period of 30 min, and the mixture was heated at 155°C for 5 hr. The charcoal brown product was then fractionally distilled under aspirator pressure; fractions between 127 and 144°C, which were collected and combined, yielded 0.28 g. To obtain pure DMP, the fraction was purified with HPLC (Shimadzu LC-20AD HPLC system; Shimadzu Corporation, Kyoto, Japan). HPLC purification was performed on a reverse-phase column with endcapping (C18; particle size, 5 μM; pore size, 100 Å; column length, 250 mm; i.d., 20 mm; Inertsil ODS-3; GL Sciences, Inc., Tokyo, Japan) employing the same C18 packing guard column (column length, 50 mm; i.d., 20 mm; GL Sciences, Inc.). The mobile phase comprised acetonitrile/water (70/30, v/v) in an isocratic mode for 45 min, and the solvent flow rate was 8 ml/min. The fractions containing DMP were collected (the elution time was 15–17 min), and the solvent was evaporated to yield a brown liquid (0.034 g) whose purity was 98% (GC). The product was characterized by nuclear magnetic resonance (NMR) spectroscopy (ECA-500 spectrometer, JEOL Ltd., Tokyo, Japan) and high-resolution (HR) fast atom bombardment (FAB)/MS (FAB + MS; JMS-700 instrument, JEOL Ltd.). NMR spectra were observed in chloroform-*d* solution, and all of the chemical shifts were obtained in parts per million downfield from tetramethylsilane (TMS). Details of NMR spectra and FAB/MS data were as follows. ^{1}H-NMR: δ 6.59 (singlet, 2 H, Ar-*H* × 2), 4.72 (singlet, 1 H, -O*H*), 2.50 (singlet, 6 H, -CH_3 × 2), 2.16 (singlet, 3 H, -SCH_3). ^{13}C-NMR: δ 155.3 (*C*-OH), 144.8 (*C*-CH_3), 126.6 (*C*-SCH_3), 115.0 (*C*-H), 21.9 (-*C*H_3), 18.8 (-S*C*H_3). HR FAB + MS mode (M^+): 168.0350 (intensity, 33%; calculated for 168.0609; $C_9H_{12}OS$).

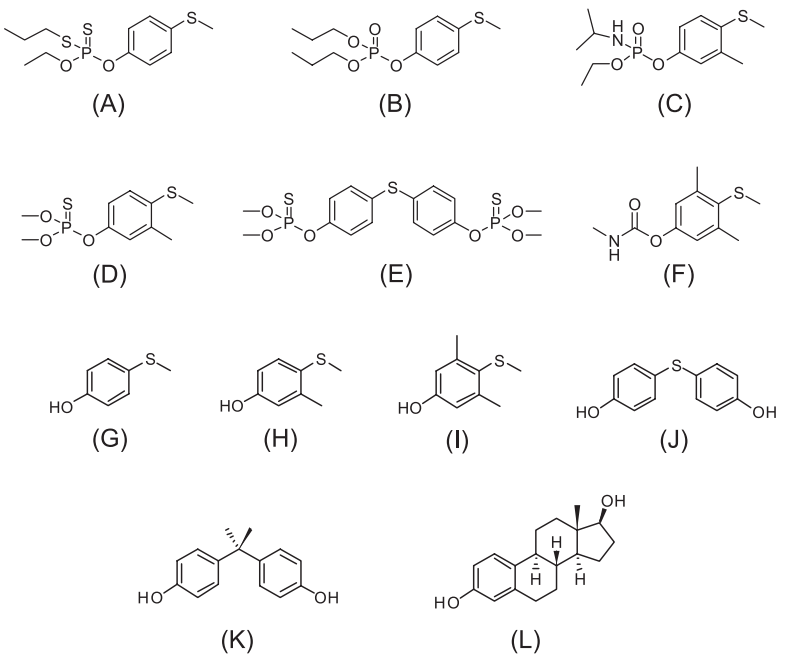


Fig. 1. Chemical Structure of the Test Compounds

(A) sulprofos, (B) propaphos, (C) fenamiphos, (D) fenthion, (E) temephos, (F) methiocarb, (G) MTP, (H) MMP, (I) DMP, (J) TDP, (K) BPA, (L) 17β-estradiol (E_2).

Yeast Two-hybrid Assay for hERα —— The estrogenic activities of the test compounds were examined with the yeast two-hybrid estrogenicity assay system developed by Nishikawa *et al.* and modified using yeast cells (*Saccharomyces cervisiae* Y190); hERα and coactivator TIF2 had been introduced into this system.[31] The expression plasmids for the hormone receptor ligand binding domain and pGAAD424-TIF-2 were introduced into the yeast cells that carried the β-galactosidase reporter gene. The assay was adapted to a chemiluminescent reporter gene (for β-galactosidase) method by employing a 96-well culture plate.[32] To measure the estrogenic activity in this system, aliquots from test chemical solutions were incubated for 4 hr at 30°C, where yeast cells were preincubated overnight at 30°C in a modified synthetic dextrose medium (not containing tryptophan and leucine). After the incubation period, a mixed solution of 2 mg/ml of zymolyase (Zymolyase 20T; Seikagaku, Tokyo, Japan) and a commercial reporter gene assay kit (Aurora Gal-XE; ICN Pharmaceuticals, Costa Mesa, CA, U.S.A.), which induces chemiluminescence and enzymatic digestion, was added at a 5 : 3 ratio. This was followed by the addition of a light-emission accelerator reagent. The intensity of the chemiluminescence produced by the released β-galactosidase was measured using a 96-well plate luminometer (Labsystems Luminoskan, Type 391A, Labsystems, Helsinki, Finland). The details of the assay have been published elsewhere.[33] The estrogenic activity was recorded as the effective concentration (EC) × 10, which is defined as the concentration of the test solution required to produce a chemiluminescent signal 10 times stronger than that of the blank control. The relative activity (RA) was calculated on the basis of the follows equation: RA (%) = (EC × 10 for BPA)/(EC × 10 for test compounds) × 100. In the present study, BPA (analytical grade) in Fig. 1K and E_2 (> 97.0% purity) in Fig. 1L were used as the reference compound and the positive control, respectively. The working range was set between 31.3–2000 pM for E_2, 313–20000 nM for BPA, 31.3–2000 nM for TDP, and 3125–200000 nM for MTP, MMP, DMP, and all other insecticides. These assays were repeated in triplicate for all of the tested compounds.

ER-ELISA —— The hERα competitive binding assay was conducted using an Estrogen-R (α) Competitor Screening kit (Wako Pure Chemical Industries, Ltd.), in accordance with the manufacturer's protocol. In brief, a mixture of fluorescent-labeled E_2 and the test compounds were added to the wells of a plate coated with hERα, and they were allowed to compete at room temperature for 2 hr. Af-

ter washing the wells with the wash-solution in the kit, the assay solution was added to each well. The fluorescence intensity in each well was read with a microplate reader (Wallac 1420 ARVOsx, Perkin-Elmer Inc., MA, U.S.A.) at an excitation and emission wavelengths of 485 and 535 nm, respectively. The inhibition curves for fluorescent-labeled E_2 binding were obtained on the basis of a reduction in the fluorescence intensity. IC_{50} values, *i.e.*, the concentrations at which replacement of fluorescent-labeled E_2 was 50%, were calculated using the inhibition curves. The relative binding ability (RBA) was calculated as follows: RBA (%) = (IC_{50} for BPA)/(IC_{50} for test compounds) × 100. In the present study, BPA (analytical grade) and E_2 (> 97.0% purity) were used as the reference compound and the positive control, respectively. The working range of the assay was set at 1.56–50 nM for E_2, 625–20000 nM for BPA, 1250–40000 nM for TDP, 12500–400000 nM for DMP, and 50000–1600000 nM for MTP and MMP. These assays were also repeated in triplicate for all of the tested compounds.

Calculations —— We calculated molecular volumes by using GaussView ver. 2.1 and Gaussian R 98W ver. 5.4.[34] The structure of a molecule was optimized in terms of a semi-empirical method with the AM1 Hamiltonian. Its volume, in an appropriate solvent replaced by a polarized continuum (PCM model), was then calculated on the basis of the B3LYP method with the 3-21G basis set.[35] All calculations were performed using a DELL Dimension 8300 micro-computer.

RESULTS AND DISCUSSION

Estrogenic Activity of Tested Compounds

First, the estrogenic potency of the *p*S-alkyl phenols, TDP and the commercial insecticides illustrated in Fig. 1 were evaluated with the yeast two-hybrid assay. Figure 2 shows the observed dose-response curves and Table 1 indicates the values for EC × 10 and RA% estimated from the curves. It was found that the activity of TDP was 10 times that of BPA, which is regarded as a significant EDC in many countries. In addition, RA% values for MTP, MMP, and DMP were estimated to be 11, 10, and 4%, respectively, of BPA activity. Although these values were smaller than that of BPA, it appeared that the compounds tested were weakly active. In addition, the EC × 10 of E_2 was estimated 0.3 ± 0.07 nM (the dose-response curve was not shown). On the other hand, dose-response relationships were not observed for all of the commercial insecticides in the range of the tested concentrations (their curves are not shown in the Fig. 2). As described above, these *p*S-alkyl phenols and TDP, which are the main degradation-products of commercial insecticides, have been regarded as safe compounds in terms of their cholinesterase-inhibiting activity. However, in terms of estrogenic activity, it should be noted that they exhibit more significant activity than insecticides themselves.

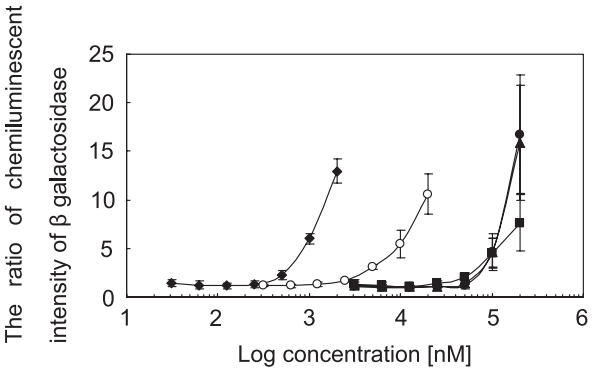


Fig. 2. Dose-response Curves in Yeast Two-hybrid Assay
●; MTP, ▲; MMP, ■; DMP, ◆; TDP, ○; BPA (reference compounds). Each point represents the mean of triplicate.

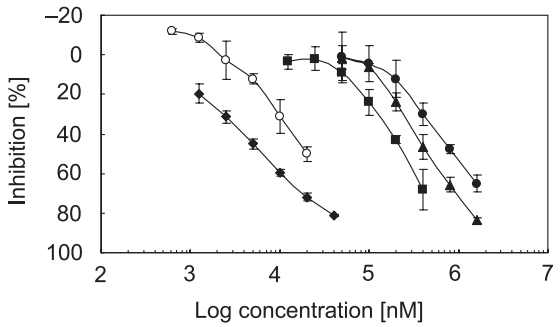


Fig. 3. Dose-response Curves in ER-ELISA
●; MTP, ▲; MMP, ■; DMP, ◆; TDP, ○; BPA (reference compounds). Each point represents the mean of triplicate.

hERα Binding Ability of Tested Compounds

Next, we carried out ER-ELISA to evaluate the binding ability of *p*S-alkyl phenols and TDP to hERα. From the dose-response curves shown in Fig. 3, the RBA% of *p*S-alkyl phenols and TDP were estimated to be in a range between 2–141% (Table 1). Regarding RBA% and RA% values, the former was smaller than the latter; for example, although the RA% of TDP was about 10 times that

Table 1. Estrogenic Activity and hERα Binding Ability of Test Compounds[a)]

Compounds	Estrogenic activity		hERα binding ability	
	EC × 10 (nM)[b)]	RA (%)[c)]	IC_{50} (nM)[d)]	RBA (%)[e)]
MTP	152000 ± 45000	11	1040000 ± 45000	2
MMP	159000 ± 54000	10	726000 ± 49000	2
DMP	449000 ± 103000	4	271000 ± 14000	6
TDP	1600 ± 150	1000	12000 ± 1200	141
BPA	17000 ± 600	100	17000 ± 700	100

a) Values are presented as the mean ± standard deviation of triplicate. *b*) EC = effective concentration; EC × 10 = the concentration of test compounds showing 10% of the activity of blank controls. *c*) RA = relative activity (BPA = 100). *d*) IC_{50} = 50% inhibition concentration. *e*) RBA = relative binding ability (BPA = 100).

of BPA (a reference compound), its RBA% was estimated to be 1.5 times of that observed with ER-ELISA. A similar lowering of the RBA% was also observed for MTP, MMP, and DMP. However, it was also found that the ordering of activities obtained from both bioassays indicated the same trend: TDP > BPA > MTP, MMP, and DMP. Therefore, we determined that *p*S-alkyl phenols and TDP possess binding ability to hERα, and that the estrogenic activity observed from the yeast two-hybrid assay is responsible for this ability. In addition, the IC_{50} of E_2 was estimated 25 ± 1 nM (the dose-response curve was not shown).

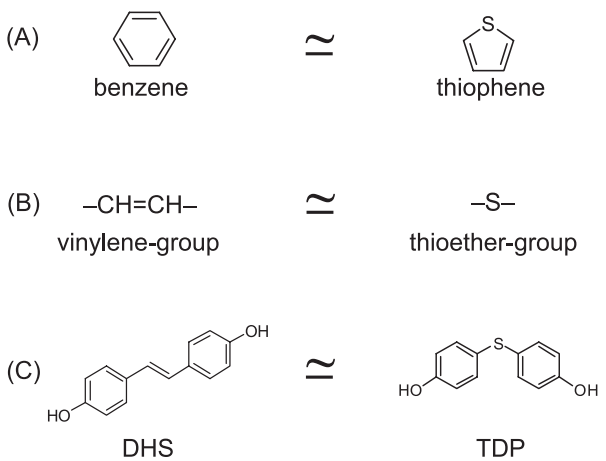


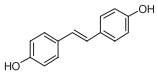
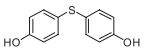
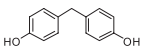
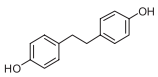
Fig. 4. Bioisosteric Relationships between Benzene and Thiophene (A), Vinylene-group and Thioether-group (B), and 4,4′-Dihydroxystilbene (DHS) and TDP (C)

An Explanation of Binding Ability on the Basis of Bioisosterism

When considering the chemical structure and estrogenic activity, it has previously been pointed out by Nishihara *et al.* that most of the positive compounds observed on the basis of the yeast two-hybrid assay possess a phenol ring with a moiety of appropriate hydrophobicity at the "*para*"-position.[17)] In medicinal chemistry, a term known as "bioisosterism," which describes functional groups related in structure and possessing similar biological effects, has been introduced.[36)] Friedman defined the terms, and Burger subsequently expanded the definition as follows: "Bioisosteres are compounds or groups that possess nearly equal molecular shapes and volumes, approximately the same distribution of electrons, and which exhibit similar physical properties such as hydrophobicity. Bioisosteric compounds affect the same biochemically associated systems as agonist or antagonists and thereby produce biological properties that are related to each other."[37,38)] Benzene and thiophene have been cited as typical examples of bioisosteric compounds. There are also many reports based on practical uses of bioisosteric replacement of one (or a portion of a) compound with another. For example, De Martino *et al.* reported that a thiophene ring is cross-superimposed with a phenyl ring by using an Autodock program package, as shown in Fig. 4A.[39)] In addition, as shown in Fig. 4B, Ghalit *et al.* reported that the conformational constraint of a thioether-part, –S–, is a similar to that of alkene or alkane.[40)]

In the present study, the *p*S-alkyl phenols and TDP can be regarded as positive compounds in terms of the estrogenic activity from the findings based on two bioassays. As a plausible reason for this positive activity, it should be noted that bioisosteric replacement between TDP and 4,4′-dihydroxystilbene (DHS) is valid, as shown in Fig. 4C. It is well known that DHS possesses estrogenic binding ability, and its potency has been estimated on the basis of an ER binding assay.[41)] Table 2 shows their molecular volumes and RBA% to hERα together with those of 4,4′-methylenediphenol (MDP) and 4,4′-ethylenediphenol (EDP).[41,42)] As regards MDP

Table 2. RBA, *n*- or π-electrons of the Bridge Moiety, and Molecular Volume in Isosteric and Non-isosteric Diphenols

Compound	Molecular structure	RBA (%)[a]	*n*- or π-electrons	Molecular volume (Å^3)
DHS		0.45[b]	○	402
TDP		0.21[c]	○	372
MDP		0.07[d]	×	344
EDP		0.06[b]	×	375

a) Relative binding ability to hERα [RBA (%) = IC_{50} of E_2/IC_{50} of test compounds × 100]. *b*) Waibel *et al.*[41] *c*) This study. *d*) Akahori *et al.*[42]

and EDP, although their molecular volumes are similar to that of TDP, they possess a methylene-group ($-CH_2-$) or an ethylene-group ($-CH_2-CH_2-$), both of which have quite different electron-distributions than a thioether-group (–S–) or a vinylene-group (–CH=CH–) in terms of the presence or absence of *n*- or π-electrons at the moieties. Judging from not only from the electron distributions of the moiety but also from the molecular volumes (Table 2), it appears that the biochemical characteristics of TDP are similar to that of DHS.

In terms of cholinesterase inhibition, *p*S-alkyl phenols and TDP have been regarded as more secure compounds than OP-CMIs or their oxon-compounds. However, an assessment of the total risk and life cycles of OP-CMIs illuminates how greater attention should be given to environmental concentrations of *p*S-alkyl phenols and TDP as well. This is underscored by the results of the present study, which found that the *p*S-alkyl phenols and TDP tested possess unintentional bioactivities such as estrogenic activity.

Acknowledgement This work was partly supported by a Grant-in-Aid for Scientific Research (No. 19051) from the Kurita Water and Environment Foundation (KWEF) to M.M.

REFERENCES

1) Temple, A. W. and Smith, A. N. (1989, Updated 1998) *Organophosphorus pesticides, International Programme on Chemical Safety (IPCS) Poisons Information Monograph G001*, IPCS. Available online at: http://www.inchem.org/documents/pims/chemical/pimg001.htm
2) Ecobichon, D., El-Sebae, H. A., Ivanova-Chemishanska, L., Johnson, K. M., Kashyap, K. S., Lotti, M., L. Martson, L, Oleru, G. U., Phoon, O. W., Rahde, F. A., Reiner, E. and Sekizawa, J. (1986) *Environmental health criteria 64, carbamate pesticides: a general introduction*, IPCS. Available online at: http://www.inchem.org/documents/ehc/ehc/ehc64.htm
3) Fernandez, M., Pico, Y. and Manes, J. (2000) Determination of carbamate residues in fruits and vegetables by matrix solid-phase dispersion and liquid chromatography-mass spectrometry. *J. Chromatogr. A*, **871**, 43–56.
4) Konstantinou, I. K., Hela, D. G. and Albanis, T. A. (2006) The status of pesticide pollution in surface waters (rivers and lakes) of Greece. Part I. Review on occurrence and levels. *Environ. Pollut.*, **141**, 555–570.
5) Tsuda, T., Nakamura, T., Inoue, A. and Tanaka, K. (2009) Pesticides in water and sediment from littoral area of lake Biwa. *Bull. Environ. Contam. Toxicol.*, **82**, 683–689.
6) Hanazato, T. (1998) Response of a zooplankton community to insecticide application in experimental ponds: a review and the implications of the effects of chemicals on the structure and functioning of freshwater communities. *Environ. Pollut.*, **101**, 361–373.
7) Gruber, S. J. and Munn, M. D. (1998) Organophosphate and carbamate insecticides in agricultural waters and cholinesterase (ChE) inhibition in common carp (Cyprinus carpio). *Arch. Environ. Contam. Toxic.*, **35**, 391–396.
8) Costa, L. G. (2006) Current issues in organophosphate toxicology. *Clin. Chim. Acta*, **366**, 1–13.
9) Slotkin, T. A., MacKillop, E. A., Ryde, I. T., Tate, C. A. and Seidler, F. J. (2007) Screening for developmental neurotoxicity using PC12 cells: com-

parisons of organophosphates with a carbamate, an organochlorine, and divalent nickel. *Environ. Health Perspect.*, **115**, 93–101.
10) Fukuto, T. R. (1990) Mechanism of action of organophosphorus and carbamate insecticides. *Environ. Health Perspect.*, **87**, 245–254.
11) Sakellarides, T. M., Siskos, M. G. and Albanis, T. A. (2003) Photodegradation of selected organophosphorus insecticides under sunlight in different natural waters and soils. *Int. J. Environ. Anal. Chem.*, **83**, 33–50.
12) Wheelock, C. E., Shan, G. and Ottea, J. (2005) Overview of carboxylesterases and their role in the metabolism of insecticides. *J. Pestic. Sci.*, **30**, 75–83.
13) Gohre, K. and Miller, G. C. (1986) Photooxidation of thioether pesticides on soil surfaces. *J. Agric. Food Chem.*, **34**, 709–713.
14) Minelli, E. V., Cabras, P., Angioni, A., Garau, V. L., Melis, M., Pirisi, F. M., Cabitza, F. and Cubeddu, M. (1996) Persistence and metabolism of fenthion in orange fruit. *J. Agric. Food Chem.*, **44**, 936–939.
15) Kavlock, R. J., Daston, G. P., DeRosa, C., Fenner-Crisp, P., Gray, L. E., Kaattari, S., Lucier, G., Luster, M., Mac, M. J., Maczka, C., Miller, R., Moore, J., Rolland, R., Scott, G., Sheehan, D. M., Sinks, T. and Tilson, H. A. (1996) Research needs for the risk assessment of health and environmental effects of endocrine disruptors: A report of the US EPA-sponsored workshop. *Environ. Health Perspect.*, **104**, 715–740.
16) Kitamura, S., Yoshida, M., Sugihara, K., Ohta, S. and Hara, A. (1999) Effect of fenthion on the level of vitellogenin in goldfish, Carassius auratus. *J. Health Sci.*, **45**, 262–265.
17) Nishihara, T., Nishikawa, J., Kanayama, T., Dakeyama, F., Saito, K., Imagawa, M., Takatori, S., Kitagawa, Y., Hori, S., and Utsumi, H. (2000) Estrogenic activities of 517 chemicals by yeast two-hybrid assay. *J. Health Sci.*, **46**, 282–298.
18) Sohoni, P., Lefevre, P. A., Ashby, J. and Sumpter, J. P. (2001) Possible androgenic/anti-androgenic activity of the insecticide fenitrothion. *J. Appl. Toxicol.*, **21**, 173–178.
19) Kojima, H., Katsura, E., Takeuchi, S., Niiyama, K. and Kobayashi, K. (2004) Screening for estrogen and androgen receptor activities in 200 pesticides by *in vitro* reporter gene assays using Chinese hamster ovary cells. *Environ. Health Perspect.*, **112**, 524–531.
20) Margni, M., Rossier, D., Crettaz, P. and Jolliet, O. (2002) Life cycle impact assessment of pesticides on human health and ecosystems. *Agriculture, Ecosystems & Environment*, **93**, 379–392.
21) Geisler, G., Hellweg, S., Liechti, S. and Hungerbuehler, K. (2004) Variability assessment of groundwater exposure to pesticides and its consideration in life-cycle assessment. *Environ. Sci. Technol.*, **38**, 4457–4464.
22) Bavcon, K. M., Franko, M. and Trebse, P. (2007) Photodegradation of organophosphorus insecticides — Investigations of products and their toxicity using gas chromatography-mass spectrometry and AChE-thermal lens spectrometric bioassay. *Chemosphere*, **67**, 99–107.
23) Caceres, T., Megharaj, M. and Naidu, R. (2008) Toxicity and transformation of fenamiphos and its metabolites by two micro algae Pseudokirchneriella subcapitata and Chlorococcum sp. *Sci. Total Environ.*, **398**, 53–59.
24) Blinn, R. C. (1969) Metabolic fate of Abate insecticide in the rat. *J. Agric. Food Chem.*, **17**, 118–122.
25) Eichelberger, J. W. and Lichtenberg, J. J. (1971) Persistence of pesticides in river water. *Environ. Sci. Technol.*, **5**, 541–544.
26) Ivie, G. W. and Bull, D. L. (1976) Photodegradation of O-ethyl O-[4-(methylthio)phenyl] S-propyl phosphorodithioate (BAY NTN 9306). *J. Agric. Food Chem.*, **24**, 1053–1057.
27) Koshioka, M., Kanazawa, J. and Murai, T. (1986) Photodegradation of an insecticide, diisopropyl 4-(methylthio)phenyl phosphate, by xenon lamp. *J. Pestic. Sci.*, **11**, 557–562.
28) Megharaj, M., Singh, N., Kookana, R. S., Naidu, R. and Sethunathan, N. (2003) Hydrolysis of fenamiphos and its oxidation products by a soil bacterium in pure culture, soil and water. *Appl. Microbiol. Biotechnol.*, **61**, 252–256.
29) Torrisi, S. and Sortino, S. (2004) New insights into the photoreactivity of the organophosphorus pesticide fenthion: a σ aryl cation as a key intermediate in the photodecomposition. *J. Agric. Food Chem.*, **52**, 5943–5949.
30) Farah, B. S. and Gilbert, E. E. (1963) Alkylthiophenols by sulfenylation of phenols. *J. Org. Chem.*, **28**, 2807–2809.
31) Nishikawa, J., Saito, K., Goto, J., Dakeyama, F., Matsuo, M. and Nishihara, T. (1999) New screening methods for chemicals with holmonal activities activities using interaction of nuclear hormone receptor with coactivator. *Toxicol. Appl. Pharmacol.*, **154**, 76–83.
32) Shiraishi, F., Shiraishi, H., Nishikawa, J., Nishihara, T. and Morita, M. (2000) Development of a simple operational estrogenicity assay system using the yeast two-hybrid system. *J.*

Environ. Chem., **10**, 7–64.
33) Shiraishi, F., Okumura, T., Nomachi, M., Serizawa, S., Nishikawa, J., Edmonds, J. S., Shiraishi, H. and Morita, M. (2003) Estrogenic and thyroid hormone activity of a series of hydroxy-polychlorinated biphenyls. *Chemosphere*, **52**, 33–42.
34) Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., Zakrzewski, V. G., Montgomery, J. A., Jr., Stratmann, R. E., Burant, J. C., Dapprich, S., Millam, J. M., Daniels, A. D., Kudin, K. N., Strain, M. C., Farkas, O., Tomasi, J., Barone, V., Cossi, M., Cammi, R., Mennucci, B., Pomelli, C., Adamo, C., Clifford, S., Ochterski, J., Petersson, G. A., Ayala, P. Y., Cui, Q., Morokuma, K., Salvador, P., Dannenberg, J. J., Malick, D. K., Rabuck, A. D., Raghavachari, K., Foresman, J. B., Cioslowski, J., Ortiz, J. V., Baboul, A. G., Stefanov, B. B., Liu, G., Liashenko, A., Piskorz, P., Komaromi, I., Gomperts, R., Martin, R. L., Fox, D. J., Keith, T., A. l-Laham, M. A., Peng, C. Y., Nanayakkara, A., Challacombe, M., Gill, P. M. W., Johnson, B., Chen, W., Wong, M. W., Andres, J. L., Gonzalez, C., Head-Gordon, M., Replogle, E. S. and Pople, J. A. (1998) *Gaussian 98*, Gaussian, Inc., Pittsburgh, PA.
35) Barone, V., Cossi, M. and Tomasi, J. (1997) A new definition of cavities for the computation of solvation free energies by the polarizable continuum model. *J. Chem. Phys.*, **107**, 3210–3221.
36) Lemke, T. L. and Williams, D. A.; Editors. (2008) Functional group modification: Isosterism and bioisosterism. In *Foye's Principles of Medicinal Chemistry*, 6th Ed., Lippincott Williams & Wilkins, Philadelphia, pp. 48–53.
37) Friedman, H. L. (1951) Influence of isosteric replacement upon biological activity. In *symposium on chemical-biological correlation*, Publication No. 206, National Academy of Sciences, U.S. Government Printing Office, Washington D.C., pp. 295–358.
38) Burger, A. (1991) Isosterism and bioisosterism in drug design. *Prog. Drug Res.*, **37**, 287–371.
39) De Martino, G., La Regina, G., Di Pasquali, A., Ragno, R., Bergamini, A., Ciaprini, C., Sinistro, A., Maga, G., Crespan, E., Artico, M. and Silvestri, R. (2005) Novel 1-[2-(Diarylmethoxy)ethyl]-2-methyl-5-nitroimidazoles as HIV-1 Non-Nucleoside Reverse Transcriptase Inhibitors. A Structure-Activity Relationship Investigation. *J. Med. Chem.*, **48**, 4378–4388.
40) Ghalit, N., Reichwein, J. F., Hilbers, H. W., Breukink, E., Rijkers, D. T. S. and Liskamp, R. M. J. (2007) Synthesis of bicyclic alkene-/alkane-bridged nisin mimics by ring-closing metathesis and their biochemical evaluation as lipid II binders: toward the design of potential novel antibiotics. *Chem. Bio. Chem.*, **8**, 1540–1554.
41) Waibel, M., De Angelis, M., Stossi, F., Kieser, K. J., Carlson, K. E., Katzenellenbogen, B. S. and Katzenellenbogen, J. A. (2009) Bibenzyl- and stilbene-core compounds with non-polar linker atom substituents as selective ligands for estrogen receptor beta. *Eur. J. Med. Chem.*, **44**, 3412–3424.
42) Akahori, Y., Nakai, M., Yamasaki, K., Takatsuki, M., Shimohigashi, Y. and Ohtaki, M. (2008) Relationship between the results of in vitro receptor binding assay to human estrogen receptor α and in vivo uterotrophic assay: Comparative study with 65 selected chemicals. *Toxicol. In Vitro*, **22**, 225–231.