JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 1549–1554

Received April 27, 2001
Accepted July 10, 2001
ISSN 0022-3573

Response of MCF-7 human breast cancer cells to some binary mixtures of oestrogenic compounds in-vitro

Takahiro Suzuki, Kunihito Ide and Masaru Ishida

Abstract

The effects of simultaneous administration of some binary mixtures of seven natural and synthetic oestrogenic substances (17β-estradiol, estrone, bisphenol A, butylbenzyl phthalate, endosulfan, methoxychlor and pentachlorophenol) on the cellular proliferation of human breast cancer MCF-7 cells in-vitro (a modified E-screen assay) have been measured. To assess the presence or absence of interactions of the two agents, the data were analysed on the basis of a graphical method in which the types and extents of interactions were described by response-surface diagrams. Of the nine combinations of the agents examined, synergistic interaction was evident for the combination of 17β-estradiol and bisphenol A, whereas the remaining eight combinations were weakly synergistic, additive and/or weakly antagonistic in the dose-range tested.

Introduction

Endocrine disruption by environmental chemicals against man and wildlife species has received widespread scientific and public concern (Colborn 1995). Such endocrine disruptors (xenoestrogens or oestrogen mimics) are suspected of promoting testicular and breast cancer (Sonnenschein & Soto 1998; Wiseman 1999), reproductive tract disorders, and a reduction in reproductive fitness (Zacharewski 1997). There are many suspected environmental endocrine disruptors in the environment and interactions among widely used chemicals are one of the most critical problems to be addressed (Arnold et al 1997). It has been discovered that some combinations of two or three xenoestrogens can be many times more potent than either chemical alone in a synergistic manner (Keith 1997). However, so far, only a limited amount of information on interactions among oestrogenic compounds has been available. In addition, the quantitative analysis of combination effects, which have been developed during the last 100 years in pharmacology and related fields, has not yet been performed (Kortenkamp & Altenburger 1998).

The aim of this study was to examine interactions of dyadic combinations of some natural and synthetic oestrogenic compounds and analyse the types of interactions. In a previous study (Suzuki et al 1998), we examined the effects of simultaneous administration of some dyadic combinations of antioxidants or vitamins and related agents on cellular proliferation of mouse leukaemia L5178Y cells in-vitro. The combined action was analysed by an approach showing the type and degree of interactions by response-surface diagrams. The mechanism-free

Chemical Resources Laboratory, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 226-8503, Japan

Takahiro Suzuki, Kunihito Ide, Masaru Ishida

Correspondence: T. Suzuki, Chemical Resources Laboratory, Tokyo Institute of Technology, 4259 Nagatuta, Midori-ku, Yokohama 226-8503, Japan. E-mail: tsuzuki@res.titech.ac.jp

Acknowledgement and Funding: We acknowledge Professor E. Metcalfe (School of Chemical and Life Sciences, University of Greenwich, London, UK) for his helpful suggestions. This work was supported by the Nippon Life Insurance Foundation, Osaka, Japan.

method required a relatively small number of experimental data compared with the conventional isobologram method or other approaches (Loewe 1953; Chou & Talalay 1984; Pei et al 1997).

Materials and Methods

Cell line

Oestrogen receptor-positive MCF-7 human breast cancer cell line JCRB0134 (Japan Health Sciences Foundation, Osaka, Japan) was used. Cells were maintained in culture flasks containing Dulbecco's modified Eagle's medium (DMEM including phenol red; BIO Whittaker, MD) supplemented with 10% (v/v) fetal bovine serum (ICN Biomedicals Inc., OH) and 1% penicillin/-streptomycin (ICN Biomedicals, Inc., OH) at 37°C in an atmosphere of 5% CO_2/95% air under saturating humidity.

Test chemicals

Commercial extra-graded reagents, 17β-estradiol (purity; $\geqslant$ 97.0%), estrone ($\geqslant$ 98.0%), bisphenol A ($>$ 95.0%), butylbenzyl phthalate ($>$ 99.0%), endosulfan (99.4%), methoxychlor ($>$ 97.0%) and pentachlorophenol (99.0%) were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). They were selected as representative agents based on the classification of 60 oestrogenic compounds by their physicochemical properties (Suzuki et al 1999, 2001).

All chemicals in dimethylsulfoxide (DMSO) were stored at 4°C and added to culture media, phenol red-free DMEM (ICN Biomedicals Inc., OH) supplemented by 10% (v/v) fetal bovine serum. Just before use the fetal bovine serum was treated with dextran (Pharmacia-LKB, Uppsala, Sweden) -coated charcoal (Norit A, acid washed; Sigma Chemical Co, St Louis, MO) to remove all steroids (Stanley et al 1977).

The concentration of DMSO in test cultures never exceeded the predetermined level of 0.1% (w/w), a concentration which did not affect cell proliferation. An equivalent amount of DMSO was added to all control cultures.

Modified E-screen assay with MCF-7 cells

The E-screen assay was carried out, with slight modifications to the original protocol (Soto et al 1995). The assay was performed in a 96-well tissue culture microtitre plate (Coaster, Cambridge, MA). The successful use of 96-well plates in E-screen assay has been reported (Körner et al 1999). Six days later the assay was terminated during the late exponential phase of proliferation and the number of cells in each well was assessed by the AlamarBlue assay (Lancaster & Fields 1996), by measuring absorbance using an automated microplate reader (Multiskan MS-UV; Labsystems, Helsinki, Finland) at wavelengths of 570 nm and 600 nm. There was a reproducible linear relationship between the measured extinction coefficient at 570 nm and the number of cells in the range of 1×10^5–1×10^6 cells mL^{-1}. This colorimetric assay was much faster and easier to perform than direct counting of cells.

Statistical analysis

Analysis of variance with Microsoft Excel was used to determine whether there were significant differences ($P < 0.05$) in cell proliferation or final cell concentrations between treated and control cells. The response surface modelling of the interaction of two agents was performed using a statistical software program MINITAB v13 (Minitab, Inc., State College, PA).

Results and Discussion

Response of MCF-7 cells to a single agent

The activity of the seven single agents capable of binding to oestrogen receptor α (Waller et al 1996) was assayed against MCF-7 cells in-vitro. The data on the dose–response curves for the tested chemicals are summarized in Figure 1. All agents tested caused significant effects ($P < 0.05$) on the cellular proliferation or assay endpoints

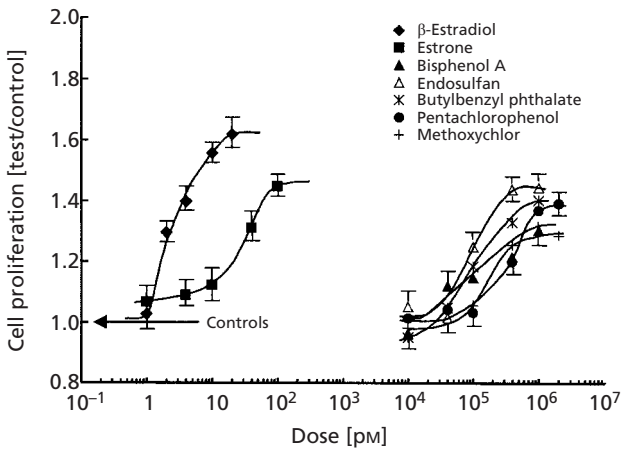

Figure 1 Oestrogenic activities of seven xenoestrogens in the MCF-7 cell line in-vitro. Results are expressed by the ratio of the number of cells in the test and control samples. Each chemical (data points) was tested at least in three independent experiments each carried out in triplicate.

from the negative control. Although these may have been specific to the conditions of the study (e.g. strain of cell line, medium and/or serum, culture flask) as for the other bioassay data, the relative potencies and effective dose ranges for the compounds compared well with those reported previously (Soto et al 1995; Balaguer et al 1999).

The most potent proliferative chemical against this cell line was 17β-estradiol (a positive control) with estrone a close second. The maximum proliferation in our system was achieved at a 17 β-estradiol dose of 2×10^{-11} M. The potencies of the other five man-made agents were 1/10 000 to 1/100 000 that of the two natural oestrogens 17β-estradiol and estrone, and their dose–response curves were concentrated in a limited domain.

The relative low potencies of each of these synthetic compounds might suggest that these chemicals alone were unlikely to produce deleterious effects on man or wildlife. However, these compounds occur as mixtures in the environment and in organisms. The possible bioaccumulating potency of many of these xenoestrogens has to be taken into account because of their high lipophilicity. In the UK, it was reported that natural oestrogens such as 17β-estradiol or estrone were dominant in sewage treatment plant effluents (Desbrow et al 1998). Therefore from a practical viewpoint, the interactions of natural steroidal oestrogens with synthetic chemicals should be considered.

Interaction of binary mixtures

The combined effects of nine pairs of agents; 17β-estradiol with six other agents (pentachlorophenol, methoxychlor, endosulfan, butylbenzyl phthalate, estrone, and bisphenol A), estrone with bisphenol A, butylbenzyl phthalate with endosulfan, and methoxychlor with pentachlorophenol, are presented in Table 1. A comparatively low concentration for each agent was adopted in this combination study, because use at or near the concentrations that produced maximal activity may result in saturable effect.

The cell proliferation effect was evaluated as the ratio of the number of cells in the test sample to those in the control sample, as shown in Figure 1. The results revealed that the concentration or the ratio of agents were important determinants in modulating responses with mixtures of chemicals as well as the combinations of agents. Although a synergistic cell proliferation could be seen with some dose combinations of 17β-estradiol plus pentachlorophenol and 17β-estradiol plus bisphenol A, a graphical method for assessing the interactions of the binary mixtures of agents would be useful.

Evaluation of interaction

To assess the interactions between two agents a graphical method was used to analyse the data shown in Table 1 (Suzuki et al 1998). Since the original method was for treating cell growth inhibition, a modification approach was used.

The synergistic index SI was defined as $SI = E_{A+B}(obsd)/E_{A+B}(cald)$, where $E_{A+B}(obsd)$ was the observed or real effect of A + B and $E_{A+B}(cald)$ was the calculated or expected effect of A + B on the basis of the effects of the individual agents. When synergism (potentiation) occurred, $SI > 1$; when additivity or zero interaction occurred, $SI = 1$; and in other cases, including antagonism, $SI < 0$.

In this study, it was postulated that the expected effect of $E_{A+B}(cald)$ could be expressed based on the effect-multiplication criterion (Eppstein & Marsh 1984; Connell et al 1985; Veckenstedt et al 1987), where the combined effect of two agents was thought to be the product of the effects of the single compounds, using the cell proliferation rate. For example, a cell proliferation rate of 1.2 for both agents would give, according to effect-multiplication, a value of 1.44 ($= 1.2^2$) for $E_{A+B}(cald)$. The response SI could be considered as a function of the doses of two agents and the unknown response function could be postulated as continuous and multiply differentiable with respect to the experimental variables in the explored domain. Under these conditions, the interrelationship between SI and doses was established through a response surface model of a quadratic polynomial (Suzuki et al 1998; Ezure & Suzuki 2001).

The contour plots generated from the analysis of the data in Table 1 are represented in Figure 2, where the doses of the two agents have been given on the x- and y-axes, and SI values were described by contour lines, respectively. Only three strongly interacting combinations, β-estradiol with pentachlorophenol (Figure 2A), butylbenzyl phthalate (Figure 2B) and bisphenol A (Figure 2C) are shown. The contour plots were made from the following quadratic response surface models by multiple regression analysis:

β-estradiol (d_A) + pentachlorophenol (d_B);

$$SI = -0.0499\, d_A + 0.00133\, d_B - 4.1 \times 10^{-5}\, d_A\, d_B + 0.00211\, d_A^2 + 5.0 \times 10^{-7}\, d_B^2 + 1.07 \quad (1)$$

$n = 16$, $r = 0.814$, $s = 0.21$

β-estradiol (d_A) + butylbenzyl phthalate (d_B);

$$SI = -0.0802\, d_A - 0.00108\, d_B + 1.0 \times 10^{-6} d_A d_B + 0.00559\, d_A^2 + 1.9 \times 10^{-6}\, d_B^2 + 0.91 \quad (2)$$

$n = 16$, $r = 0.909$, $s = 0.05$

Table 1 Cell proliferation of simultaneous administration of dyadic combinations of xenoestrogens in the MCF-7 cell line in-vitro.

	β-Estradiol (pM)				
Pentachlorophenol (nM)	0	1	2	4	10
0	1.00±0.04	1.02±0.06	1.25±0.10	1.40±0.10	1.55±0.11
10	1.01±0.04	1.08±0.06	0.98±0.09	1.33±0.10	1.30±0.13
40	1.02±0.06	1.27±0.08	1.36±0.06	1.04±0.13	1.54±0.12
100	1.13±0.05	1.46±0.10	1.33±0.05	1.35±0.20	1.16±0.11
400	1.20±0.06	1.57±0.12	2.47±0.15	2.97±0.30	2.12±0.18
	β-Estradiol (pM)				
Methoxychlor (nM)	0	1	2	4	10
10	1.01±0.05	0.99±0.05	1.06±0.06	1.42±0.08	0.67±0.08
40	1.02±0.05	0.82±0.05	0.96±0.05	1.64±0.11	0.64±0.07
100	1.11±0.05	0.73±0.06	0.65±0.05	0.66±0.05	0.79±0.05
400	1.25±0.06	0.68±0.05	0.75±0.05	0.72±0.05	1.00±0.05
	β-Estradiol (pM)				
Endosulfan (nM)	0	1	2	4	10
10	1.02±0.05	0.95±0.05	0.98±0.04	0.99±0.05	1.11±0.05
40	1.03±0.05	0.62±0.28	0.94±0.07	0.97±0.05	1.36±0.08
100	1.25±0.06	1.08±0.06	1.23±0.09	1.16±0.06	1.41±0.11
400	1.44±0.08	1.21±0.09	1.18±0.06	1.29±0.10	1.43±0.07
	β-Estradiol (pM)				
Butylbenzyl phthalate (nM)	0	1	2	4	10
10	0.96±0.05	0.89±0.05	0.86±0.05	0.79±0.05	0.97±0.05
40	1.04±0.05	0.80±0.06	0.95±0.05	0.97±0.06	1.05±0.05
100	1.18±0.05	0.88±0.05	0.98±0.05	1.03±0.06	1.02±0.05
400	1.32±0.06	0.94±0.06	1.08±0.06	1.08±0.06	1.11±0.05
	β-Estradiol (pM)				
Estrone (pM)	0	1	2	4	10
1	1.06±0.05	0.84±0.05	0.82±0.06	0.83±0.05	1.17±0.05
4	1.09±0.05	0.73±0.05	0.76±0.05	0.77±0.10	0.88±0.12
10	1.13±0.06	0.74±0.06	0.86±0.05	1.27±0.15	1.48±0.11
40	1.31±0.06	1.73±0.10	1.74±0.13	1.75±0.11	1.13±0.07
	β-Estradiol (pM)				
Bisphenol A (nM)	0	1	2	4	10
10	0.98±0.04	1.13±0.06	1.10±0.08	0.88±0.10	0.75±0.09
40	1.05±0.04	0.77±0.06	0.95±0.15	1.73±0.13	1.14±0.11
100	1.15±0.05	1.44±0.11	3.31±0.25	2.44±0.15	2.21±0.25
400	1.22±0.06	3.40±0.38	3.88±0.41	5.89±0.35	2.34±0.24
	Bisphenol A (nM)				
Estrone (pM)	0	10	40	100	400
1	1.06±0.04	0.86±0.04	0.70±0.05	0.79±0.03	0.80±0.05
4	1.09±0.05	0.87±0.05	0.95±0.05	0.82±0.04	0.82±0.10
10	1.12±0.06	0.93±0.05	0.94±0.05	0.98±0.05	0.96±0.07
40	1.31±0.08	0.87±0.08	0.93±0.06	0.93±0.05	1.12±0.06
	Endosulfan (nM)				
Butylbenzyl phthalate (nM)	0	10	40	100	400
10	1.02±0.05	0.94±0.05	0.96±0.05	0.92±0.05	0.97±0.05
40	1.02±0.05	0.86±0.05	0.92±0.06	1.10±0.04	1.26±0.05
100	1.25±0.05	1.01±0.05	1.01±0.05	0.93±0.05	1.01±0.06
400	1.44±0.07	0.99±0.05	0.98±0.05	1.24±0.05	1.32±0.08
	Pentachlorophenol (nM)				
Methoxychlor (nM)	0	10	40	100	400
10	1.01±0.05	1.16±0.04	1.18±0.05	1.10±0.05	1.13±0.05
40	1.01±0.05	1.19±0.07	1.11±0.05	1.13±0.06	1.08±0.07
100	1.06±0.05	1.22±0.10	1.30±0.08	1.29±0.06	1.26±0.08
400	1.25±0.06	1.15±0.04	1.21±0.05	1.22±0.06	1.20±0.08

Results are expressed as the ratio of the number of cells in the test and control samples (mean±s.e.m.). Each value is the mean of three independent experiments with three replicates.

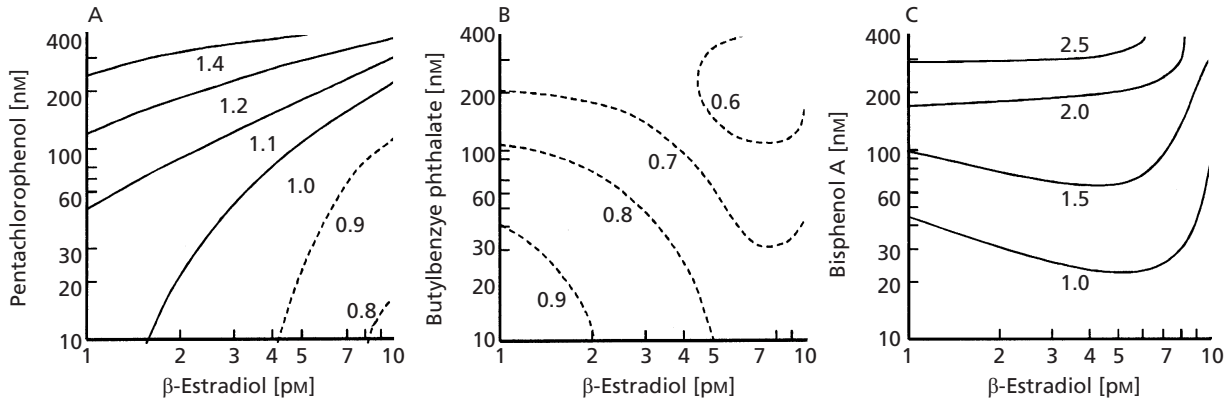

Figure 2 Contour plots showing the variation of the synergistic index (SI) as a function of doses of agents of β-estradiol in combination with (A) pentachlorophenol, (B) butylbenzyl phthalate and (C) bisphenol A. SI values equal to, greater than, or smaller than unity were evaluated as zero interaction, synergism, or antagonism, respectively. Full contour lines show positive SI regions and broken lines show negative regions.

β-estradiol (d_A) + bisphenol A (d_B);

$$SI = 0.247\, d_A + 0.0124\, d_B - 3.62 \times 10^{-4} d_A d_B - 0.0234\, d_A^2 - 1.53 \times 10^{-5}\, d_B^2 + 0.24 \quad (3)$$

$n = 16$, $r = 0.916$, $s = 0.42$

In the above equations, d_A and d_B are the doses of agents A and B, n is the number of data points, r is the correlation coefficient, and s is the standard deviation. As can been seen from these equations, fairly high r-values were found in all nine combination cases. For other combinations, r-values of 0.814–0.964 and s-values of 0.05–0.2 were observed.

It should be noted that the SI values shown in the diagram were just statistically determined values with inherent uncertainty. Accordingly, for the experimental uncertainty (see Table 1) and s-values from the above treatments, an uncertainty of around 0.2 in SI should be taken into account for judging the presence or absence of synergy.

This diagram clearly showed regions of synergism, antagonism or zero interaction within the dose range under study. The projections shown in Figure 2 indicated that within the range of doses studied the combinations of 17β-estradiol with pentachlorophenol and 17β-estradiol with bisphenol A exhibited synergism as suggested in Table 1, whereas butylbenzyl phthalate appeared to antagonize the natural oestrogen. The combination methoxychlor with pentachlorophenol was a zero interaction combination (SI = 1.0–1.1) and the remaining pairs were weak antagonistic combinations. Weak synergism (SI = 1.1–1.4) between 17β-estradiol and pentachlorophenol could be observed in a limited dose combination region. For some dose combinations of 17β-estradiol and estrone, synergistic activity in yeast expressing human oestrogen receptor has been observed (Arnold et al 1997), although synergy was not observed with the mixture in this study. To the best of our knowledge, the in-vitro activities of the other eight combinations of binary mixtures examined have not been reported.

The oestrogenic effects observed were mediated by oestrogen receptor. According to the data of the crystal structure of oestrogen receptor-α (Brzozowski et al 1997), the gap of the ligand binding domain was much larger than the natural ligand 17β-estradiol required, so there was space for a variety of other molecules to interact with the receptor. A structurally-diverse assortment of 60 xenoestrogens was classified into four clusters using both principal component analysis and hierarchical cluster analysis based on their physicochemical properties, and a dendrogram showing the similarities of the agents was derived (Suzuki et al 2001). According to the dendrogram (compounds within a cluster would be expected to show a similar binding ability to oestrogen receptor), bisphenol A and estrone were included in the same cluster including 17β-estradiol and five other compounds were in other clusters. The distances from 17β-estradiol were in the order: estrone < bisphenol A < endosulfan < butylbenzyl phthalate < methoxychlor < pentachlorophenol. The closer the distance, the more similar were the mechanisms or driving forces to oestrogen-receptor binding. It might be believed that two similar agents have common binding sites in most cases. In synergistic interactions of 17β-estradiol with bisphenol A, the observed effect might suggest that the oestrogen receptor contained multiple estrogen-binding sites and that the interaction of

oestrogen receptor with two different chemicals could produce synergistic transactivation (Arnold et al 1997). It also seemed likely that some agents induced the creation of extra receptor sites in the cell. Further studies are required to clarify the mechanism of interaction between 17β-estradiol and bisphenol A.

Conclusions

The modified E-screen assay was applied to test the interactions of binary mixtures from seven known oestrogenic compounds. The nature of the interactions changed with altered exposure concentrations. The combination of 17β-estradiol and bisphenol A markedly enhanced cell proliferation. The mixture produced an activity approximately 2.5-fold higher than expected at some dose combinations. The elucidation of the mechanism would be important for understanding how such combinations of natural and synthetic oestrogens could affect the cellular proliferation of MCF-7 cells in-vitro.

References

Arnold, S. F., Bergeron, J. M., Tran, D. Q., Collins, B. M., Vonier, P. M., Crews, D., Toscano, W. A. Jr., McLachlan, J. A. (1997) Synergistic responses of steroidal estrogens in vitro (yeast) and in vivo (turtles). *Biochem. Biophys. Res. Commun.* **235**: 336–342

Balaguer, P., François, F., Comunale, F., Fenet, H., Boussioux, A.-M., Pons, M., Nicolas, J.-C., Casellas, C. (1999) Reporter cell lines to study the estrogenic effects of xenoestrogens. *Sci. Total Environ.* **233**: 47–56

Brzozowski, A. M., Pike, A. C. W., Dauter, Z., Hubbard, R. E., Bonn, T., Engström, O., Öhman, L., Greene, G. L., Gustafsson, J.-Å., Carlquist, M. (1997) Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* **389**: 753–758

Chou, T. C., Talalay, P. (1984) Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv. Enzyme Regul.* **22**: 27–55

Colborn, T. (1995) Environmental estrogens: health implications for humans and wildlife. *Environ. Health Perspect.* **103** (Suppl. 7): 135–136

Connell, E. V., Cerruti, R. L., Trown, P. W. (1985) Synergistic activity of combinations of recombinant human alpha interferon and acyclovir, administered concomitantly and in sequence, against a lethal herpes simplex virus type 1 infection in mice. *Antimicrob. Agents Chemother.* **28**: 1–4

Desbrow, C., Routledge, E. L., Brighty, G. C., Sumpter, J. P., Waldock, M. (1998) Identification of estrogenic chemicals in STW effluents. 1. chemical fractionation and in vitro biological screening. *Environ. Sci. Technol.* **32**: 1549–1558

Eppstein, D. A., Marsh, Y. V. (1984) Potent synergistic inhibition of herpes simplex virus-2 by 9-[(1,3-dihydroxy-2-propoxy)methyl]-guanin in combination with recombinant interferons. *Biochem. Biophy. Res. Commun.* **120**: 66–73

Ezure, T., Suzuki, T. (2001) A response surface approach to evaluating drug-drug interactions: application to the cytotoxicity of some binary mixtures of agents against cancer cells in-vitro. *Anal. Pharmacol.* **2**: 57–61

Keith, L. H. (1997) *Environmental endocrine disruptors: a handbook of property data*. John Wiley & Sons, New York, pp 1–16

Körner, W., Hanf, V., Schuller, W., Kempter, C., Metzger, J., Hagenmaier, H. (1999) Development of a sensitive E-screen assay for quantitative analysis of estrogenic activity in municipal sewage plant effluents. *Sci. Total Environ.* **225**: 33–48

Kortenkamp, A., Altenburger, R. (1998) Synergisms with mixtures of xenoestrogens: a reevaluation using the method of isoboles. *Sci. Total Environ.* **221**: 59–73

Lancaster, M. V., Fields, R. D. (1996) Antibiotic and cytotoxic drug susceptibility assays using resazurin and poising agents. U. S. Patent No.5, 501, 959

Loewe, S. (1953) The problem of synergism and antagonism of combined drugs. *Arzneimittelforschung* **3**: 285–290

Pei, X.-H., Nakanishi, Y., Takayama, K., Bai, F., Kawasaki, M., Tsuruta, N., Mizuno, K., Hara, N. (1997) Effect of CPT-11 in combination with other anticancer agents in lung cancer cells. *Anti-Cancer Drugs* **8**: 231–237

Sonnenschein, C., Soto, A. M. (1998) An updated review of environmental estrogen and androgen mimics and antagonists. *J. Steroid Biochem. Mol. Biol.* **65**: 143–150

Soto, A. M., Sonnenschein, C., Chung, K. L., Fernandez, M. F., Olea, N., Olea-Serrano, F. (1995) The E-screen assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. *Environ. Health Perspect.* **103**: 113–122

Stanley, E. R., Palmer, R. E., Sohn, U. (1977) Development of methods for the quantitative in vitro analysis of androgen-dependent and autonomous Shionogi carcinoma 115 cells. *Cell* **10**: 35–44

Suzuki, T., Ezure, T., Ishida, M. (1998) Synergistic effects of some pairs of antioxidants and related agents on mouse leukaemia L5178Y cell growth in-vitro. *J. Pharm. Pharmacol.* **50**: 1173–1177

Suzuki, T., Ide, K., Ishida, M. (1999) Evaluation of synergistic effects of environmental oestrogens. *J. Pharm. Pharmcol.* **51** (Suppl.): 142

Suzuki, T., Ide, K., Ishida, M., Shapiro, S. (2001) Classification of environmental estrogens by physicochemical properties using principal component analysis and hierarchical cluster analysis. *J. Chem. Inf. Comput. Sci.* **41**: 718–726

Veckenstedt, A., Güttner, J., Beladi, I. (1987) Synergistic action of quercetin and murine α/β interferon in the treatment of Mengo virus infection in mice. *Antiviral Res.* **7**: 169–178

Waller, C. L., Oprea, T. I., Chae, K., Park, H.-K., Korach, K. S., Laws, S. C., Wiese, T. E., Kelce, W. R., Gray, L. E. (1996) Ligand-based identification of environmental estrogens. *Chem. Res. Toxicol.* **9**: 1240–1248

Wiseman, H. (1999) Importance of oestrogen, xenoestrogen and phytoestrogen metabolism in breast cancer risk. *Biochem. Soc. Trans.* **27**: 299–304

Zacharewski, T. (1997) In vitro bioassays for assessing estrogenic substances. *Environ. Sci. Technol.* **31**: 613–623