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Original Article

Evaluation of the Vitotox™ test as a high-throughput genotoxicity assay

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Summary

The Vitotox™ test is a high-throughput bacterial genotoxicity test based on the SOS DNA-repair system induced by genotoxic compounds. Two genetically engineered *Salmonella typhimurium* strains are used in this system, TA104*recN*2-4 (Genox strain), that contains the bacterial luciferase (*lux*) operon (*luxCDABE*) under transcriptional control of *recN* promoter, and TA104 *pr1* (Cytox strain), that constitutively expresses *lux* operon.

The performance of the Vitotox™ test was evaluated with 33 known Ames positive chemicals, 26 known Ames negatives and 18 drug candidates developed at Mitsubishi Pharma Corporation. Ten compounds had inconclusive results because they caused SOS-independent enhancement of light emission. Among 49 known chemicals with conclusive results, 89% of the Ames positive compounds were detected as positive (genotoxic) with the Vitotox™ test, and all of the Ames negative compounds were detected as negative. There was a 94% concordance between the Ames test results and the Vitotox™ test results.

In a practical validation study using 18 drug candidates developed at Mitsubishi Pharma Corporation, 7 of 8 Ames positive compounds were detected as genotoxic and all of the Ames negative compounds gave negative results with the Vitotox™ test. The concordance between the Vitotox™ test results and the Ames test results for 18 drug candidates was 94% (17/18). Moreover, the Vitotox™ test required a smaller sample quantity than the Ames test to detect genotoxicity.

The present results indicate that the Vitotox™ test is useful for rapid screening of large numbers of chemicals when only a small quantity of a chemical is available.

Keywords: Vitotox™ test, SOS response, genotoxicity, high-throughput system

Introduction

Recently, new technologies such as pharmacogenomics and combinatorial chemistry have accelerated research for the development of new pharmaceuticals. While the number of chemicals to be evaluated has increased, the quantity of the compound available for testing has been limited. Under such situations, a high-throughput assay is required for rapid genotoxicity screening at the early stage of drug development.

The Ames test is the most widely used as an initial screening of genotoxicity for newly synthesized chemicals (Ames et al., 1975). However, this assay has disadvantages for screening of a large number of chemicals, namely the use of time-consuming detection methods like colony counting and the need for about three days to obtain results.

Alternatively, new bacterial assays, based on the measurement of the DNA damage-dependent induction of the bacterial SOS system, such as SOS chromo test, *umu* test and *rec-lac* test, have been developed since the early 1980's (Quillardet et al., 1982; Oda et al., 1985; Nunoshiba and Nishioka, 1991). The results of the SOS chromotest and the *umu* test are comparable with those of the Ames test, showing 82% and 90% concordance, respectively (Quillardet and Hofnung, 1993; Reiffer-

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Table 1 The results of Vitotox™ test and comparison with other genotoxicity tests

Test compounds	CAS No.	Source[a]	Vitotox™ [b] (Present study) −S9	+S9	Dose range (nM)	Vitotox™ [b]	Ames[c]	SOS[d]	*umu*[e]	Carcinogenicity[c]
Ames positive compounds										
9-Aminoacridine (9-AA)	134-50-9	S	I	I	7.8×10^{2}–6.1	N.A.	+	+/−	(+)[h]	N.A.
2-Aminoanthracene (2-AA)	613-13-8	W	−	+	3.1×10–2.4×10^{-1}	N.A.	+	+	+	+
2-Aminofluorene (2-AF)	153-78-6	W	−	+	3.5×10^{2}–2.8	+	+	+	N.A.	+
2-Amino-3-methyl-3H-imidazo[4,5-*f*]quinoline (IQ)	76180-96-6	W	−	+	$6.1-4.7\times10^{-2}$	N.A.	+[f]	N.A.	+	+
2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)	105650-23-5	W	−	+	1.8×10^{2}–1.4	N.A.	+[f]	N.A.	+	+
3-Amino-1-methyl-5H-pyrido[4,3-*b*]indole (Trp-P-2)	72254-58-1	W	−	+	$2.3\times10-1.8\times10^{-1}$	N.A.	+[f]	N.A.	+	+
5-Azacytidine	320-67-2	W	+	+	$2.5-2.0\times10^{-2}$	N.A.	+	N.A.	N.A.	+
Azobenzene	103-33-3	W	−	+	2.7×10^{3}–2.1×10	N.A.	+	N.A.	(+)[h]	+
Benzo[*a*]pyrene (B[a]P)	50-32-8	T	−	+	2.5×10^{2}–2.0	+	+	+	+	+
Daunorubicin (Dau)	23541-50-6	W	+	+	$1.7\times10-1.4\times10^{-1}$	N.A.	+[g]	+	+	+
2,4-Diaminotoluene (2,4-DAT)	95-80-7	W	I	I	8.2×10^{5}–6.4×10^{2}	N.A.	+	N.A.	(+)[i]	+
2,6-Diaminotoluene (2,6-DAT)	823-40-5	T	I	I	8.2×10^{5}–6.4×10^{2}	N.A.	+	N.A.	(+)[i]	−
1,3-Dichloropropene	542-75-6	W	+	+	1.1×10^{5}–8.8×10^{2}	N.A.	+	+	N.A.	+
Diethylnitrosamine (DEN)	55-18-5	W	−	+	9.8×10^{5}–7.6×10^{2}	+	+	+	(+)[h]	+
7,12-Dimethylbenzanthracene (DMBA)	57-97-6	W	−	+	1.4×10^{2}–1.1	N.A.	+	+	+	+
1,2-Dimethylhydrazine (DMH)	57-14-7	T	I	I	7.5×10^{5}–5.9×10^{2}	N.A.	+	N.A.	+/−	+
Dimethylnitrosamine (DMN)	62-75-9	W	−	+	1.3×10^{5}–1.1×10^{3}	N.A.	+	+	+	+
1,2-Epoxybutane	106-88-7	W	−	−	1.1×10^{5}–8.7×10^{2}	N.A.	+	+	+	+
Ethidium bromide (EtBr)	1239-45-8	S	−	+	3.8×10^{2}–3.0	N.A.	+	+/−	+	+
Ethyl methanesulfonate (EMS)	62-50-0	N	+	+	8.1×10^{4}–6.3×10^{2}	+	+	+	+	+
N-Ethyl-*N*'-nitro-*N*-nitrosoguanidine (ENNG)	4245-77-6	T	+	+	$4.9\times10-3.9\times10^{-1}$	N.A.	+[g]	+	+	+
Furylfuramide (AF-2)	3688-53-7	W	+	+	3.2×10^{-1}–2.5×10^{-3}	N.A.	+	+	+	+
8-Hydroxyquinoline	148-24-3	T	−	−	6.9×10^{2}–5.4	N.A.	+	−	−	−
Hydroxyurea	127-07-1	S	I	I	1.3×10^{5}–1.0×10^{3}	N.A.	+	+	+	I
ICR191	17070-45-0	W	+	+	2.1×10^{5}–1.7×10^{2}	+	+	+	N.A.	N.A.
3-Methylcholanthrene (3-MC)	56-49-5	S	−	+	1.1×10^{2}–8.7×10^{-1}	N.A.	+	+	+	+
Methyl methanesulfonate (MMS)	66-27-3	T	+	+	5.8×10^{3}–4.5×10	+	+	+	+	+
N-Methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG)	70-25-7	S	+	+	1.6×10^{2}–1.3	N.A.	+	+	+	+
Mitomycin C (MMC)	50-07-0	W	+	+	$7.5-6.0\times10^{-2}$	+	+	N.A.	+	+
2-Nitrofluorene (2-NF)	607-57-8	S	+	+	6.0×10^{2}–4.7	N.A.	+	+	+	+
4-Nitroquinoline-*N*-oxide (4NQO)	56-57-5	W	+	+	2.6×10^{3}–2.0×10	+	+	+	+	+
N-Nitrosomethylurea (NMU)	684-93-5	S	+	+	4.9×10^{3}–3.8×10	N.A.	+	+	+	+
Styrene oxide	35311	W	−	−	$2.6\times10-2.1\times10^{-1}$	N.A.	+	+	+	+

Table 1 (continued)

Test compounds	CAS No.	Source[a)]	Vitotox™ [b)] (Present study) −S9	+S9	Dose range (nM)	Vitotox™ [b)]	Ames[c)]	SOS[d)]	*umu*[e)]	Carcinogenicity[c)]
Ames negative compounds										
Actinomycin D	50-76-0	S	−	−	$8.0\times10^{3}-6.2\times10$	N.A.	−	−	−	+
Benzoin	119-53-9	W	−	−	$4.7\times10^{4}-3.7\times10^{2}$	N.A.	−	N.A.	−	−
Bisphenol A	80-05-7	T	−	−	$4.4\times10^{4}-3.4\times10^{2}$	N.A.	−	N.A.	N.A.	+/−
Butylated hydroxyanisol	25013-16-5	W	−	−	$2.8\times10^{3}-2.1\times10$	N.A.	−	−	−	−
Caffein	58-08-2	S	−	−	$5.1\times10^{4}-4.0\times10^{2}$	N.A.	−	−	−	−
Caprolactam	105-60-2	W	I	I	$8.8\times10^{4}-6.9\times10^{2}$	N.A.	−	N.A.	−	−
Chlorodibromomethane	124-48-1	W	−	−	$4.8\times10^{4}-3.8\times10^{2}$	N.A.	−	N.A.	N.A.	−
3-Chloro-2-methylpropene	563-47-3	W	−	−	$1.1\times10^{4}-8.6\times10^{2}$	N.A.	−	N.A.	N.A.	+
Chlorpheniramine maleate	113-92-8	S	−	−	$2.6\times10^{4}-2.0\times10^{2}$	N.A.	−	N.A.	N.A.	+
Chromotrope FB	3567-69-9	S	−	−	$2.0\times10^{4}-1.56\times10^{2}$	N.A.	−	N.A.	N.A.	+
DDT	50-29-3	T	−	−	$8.8\times10^{4}-6.9\times10^{2}$	N.A.	−	−	N.A.	+
Di(2-ethylhexyl) phthalate	117-81-7	W	−	−	$2.6\times10^{4}-2.0\times10^{2}$	N.A.	−	N.A.	N.A.	+
Diethylstilbesterol	56-53-1	T	−	−	3.7×10^{4} 3.0×10^{2}	N.A.	−	N.A.	−	+
5-Fluorouracil	51-21-8	S	−	−	$1.2\times10^{3}-9.0$	N.A.	−	+/−	+	−
Isophorone	78-59-1	T	I	I	$7.2\times10^{4}-5.7\times10^{2}$	N.A.	−	N.A.	N.A.	+
d-Mannitol	69-65-8	W	I	I	$5.5\times10^{4}-4.3\times10^{2}$	N.A.	−	N.A.	−	−
dl-Menthol	15356-70-4	T	−	−	$6.4\times10^{4}-5.0\times10^{2}$	N.A.	−	−	N.A.	−
Methoxychlor	72-43-5	S	−	−	$2.9\times10^{4}-2.3\times10^{2}$	N.A.	−	N.A.	−	−
Phenobarbital	50-06-6	W	−	−	$4.3\times10^{4}-3.4\times10^{2}$	N.A.	−	N.A.	−	+
Reserpine	50-55-5	W	−	−	$1.6\times10^{4}-1.3\times10^{2}$	N.A.	−	N.A.	−	+
Saccharin sodium	128-44-9	W	−	−	$4.9\times10^{4}-3.9\times10^{2}$	N.A.	−	N.A.	−	+
Safrole	94-59-7	T	−	−	$6.2\times10^{4}-4.8\times10^{2}$	N.A.	−	−	−	+
Sulfisoxazole	127-69-5	S	−	−	$3.7\times10^{4}-2.9\times10^{2}$	N.A.	−	N.A.	N.A.	−
Thioacetamide	62-55-5	W	I	I	$1.3\times10^{4}-1.0\times10^{3}$	N.A.	−	N.A.	−	+
Urethane	51-79-6	S	I	I	$1.1\times10^{4}-8.8\times10^{2}$	N.A.	−	−	−	+
WY-14643	50892-23-4	S	−	−	$3.1\times10^{4}-2.4\times10^{2}$	N.A.	−	N.A.	N.A.	+

+, positive; −, negative; +/−, equivocal; (+), positive in the particular condition; I, inconclusive; N.A., not available; a) These componds were obtained from following sources;
N, Nacalai Tesque Inc. (Kyoto, Japan); S, Sigma-Aldrich (St. Louis, U.S.A.); T, Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan); W, Wako Pure Chemical Industries Ltd. (Osaka, Japan);
b) Verschaeve et al., 1999; c) Data from National Toxicology Program; d) Quillardet et al., 1993; e) Reifferscheid et al., 1996; f) Sugimura, 1985; g) McCann et al., 1975;
h) Nakamura et al., 1987; i) Oda et al., 1995

scheid and Heil, 1996). SOS response-based assays provide a result in a shorter period of time than the Ames test, i.e., within a day. Because of the simplicity of SOS-based genotoxicity assays, attention is being paid to these assays as candidates for high-throughput genotoxicity screening tests. Recently, efforts have been made to improve the sensitivity and simplicity of SOS response-based assays. These efforts include employment of newly developed reporter systems, such as green fluorescent protein (GFP) and luciferase expression vectors (Ptitsyn et al., 1997; Vollmer et al., 1997; Justus and Thomas, 1998; Kostrzynska et al., 2002).

The Vitotox™ test, established by van der Lelie et al., is an SOS response-based genotoxicity assay (van der Lelie et al., 1997; Verschaeve et al., 1999) and the system is now commercially available as Vitotox™ Test System. The bacterial strains used in this test are *S. typhimurium* TA104 *recN*2-4 (Genox strain) and *S. typhimurium* TA104 *pr1* (Cytox strain). The Genox strain possesses a plasmid containing *lux* operon (*luxCDABE*) of *Vibrio fischeri*, a luminous bacteria, under transcriptional control of mutated *recN* promoter (*recN*2-4). The Cytox strain possesses a plasmid containing *lux* operon, transcriptionally controlled by constitutive promoter (cloned from *Eco*RI-digested DNA fragment from *A. eutrophus* CH34), and constitutively expresses *lux* operon. Treatment of the Genox strain with a genotoxic compound activates the *recN* promoter, which results in the induction of the *lux* operon followed by the enhancement of light emission. The latter strain is used to examine cytotoxicity and is also used as the reference for nonspecific enhancement of light emission. Concomitant use of the Genox and the Cytox strains can exclude the false positive results caused by other induction mechanisms irrespective of genotoxicity.

The Vitotox™ test is considered to be a useful method because it is highly sensitive and the procedure is simple compared to the SOS chromotest (van der Lelie et al., 1997). However, the background data are limited compared to the Ames test, the SOS chromotest and the *umu* test (Verschaeve et al., 1999). The purpose of this study was a further evaluation of the Vitotox™ test as a high-throughput genotoxicity screening test for various chemicals.

Material and Methods

Test compounds

Fifty-nine chemicals, previously examined with the Ames test, were selected to validate the Vitotox™ test. These chemicals were of the highest quality commercially available and the sources of these chemicals are given in Table 1. The 33 Ames positive chemicals contain 28 rodent carcinogens, and 3 non-carcinogens, but the remaining two chemicals have no available carcinogenicity data. The 26 Ames negative chemicals contain 15 carcinogens and 11 non-carcinogens. The 18 compounds synthesized as drug candidates at Mitsubishi Pharma Corporation, for which the Ames test results were available, were also used for a practical validation study.

The bacterial strains and S9 mix

Two tester strains, *S. typhimurium* TA104 *recN*2-4 (Genox strain) and *S. typhimurium* TA104 *pr1* (Cytox strain) were supplied as components of the Vitotox™ 10 Kit, purchased from Thermo Labsystems (Vantaa, Finland). Rat liver S9, purchased from Kikkoman Co. (Chiba, Japan), was prepared from the liver homogenates of SD rats treated with phenobarbital and 5,6-benzoflavone. Co-factor I was purchased from the Oriental Yeast Co., Ltd. (Tokyo, Japan).

Test procedure

Each sample compound was dissolved in DMSO or distilled water and a 2-fold dilution series was made with the same solvent. Each preparation was then diluted 10-fold with purified water, and the resultant solutions were assayed. The doses were selected by referring to the doses used in the *umu* test or SOS chromotest (Quillardet et al., 1985; Nakamura et al., 1987). For newly synthesized chemicals, 1000 μg/mL was selected as the highest concentration. When it was not possible or technically difficult to transfer the sample preparations to the microplate (e.g., unable to pipette due to large precipitates), a lower concentration that could yield proper treatment was selected as the highest concentration. An appropriate lower concentration range was also selected when the sample showed severe cytotoxicity.

Overnight cultures of Genox and Cytox strains, in the optimal densities (0.2-0.5 for Genox strain and 0.4-0.6 for Cytox strain), were diluted 10- and 2-fold with medium, respectively.

Ten μL of the sample preparations was added to each well of a 96-well microplate. Ten μL of the S9 mix (10% v/v S9) or distilled water and 80 μL of the bacterial culture (Genox or Cytox) were also added to each well. The light production from each well was measured every 5 min for 4 hr at 30℃ using a luminometer (Fluoroskan Ascent FL, Thermo Labsystems). The data were analyzed with Ascent Software (Thermo Labsystems) to calculate the signal-to-noise ratio (S/N), i.e., the ratio between light production value from cells treated with sample compounds and light production value from corresponding vehicle-treated cells for each time point, for both Genox and Cytox strains. Then the maximum S/N ratio between 60 to 240 min for each sample concentration, and for both strains, was extracted and the ratio between the maximal S/N values of the Genox and the Cytox strains (Genox/Cytox ratio) was calculated for each sample con-

Table 2 The results of the Vitotox™ test and Ames test for newly synthesized chemicals

Test compounds	Category[a)]	Purity (%)	Highest dose (μg/mL)	Vitotox™ −S9	Vitotox™ +S9	Ames −S9	Ames +S9
1	A	99.0<	1000	+	+	+	+
2	A	99.8	250	+	+	+	+
3	A	99.5	1000	+	+	+	+
4	A	99.0<	1000	−	−	−	−
5	A	99.0<	1000	−	−	−	−
6	A	99.0	1000	−	−	−	−
7	B	99.6	1000	+	+	+	+
8	B	99.6	1000	+	+	+	+
9	B	99.0	1000	+	+	+	+
10	B	99.3	1000	−	−		−
11	B	99.1	1000	−	−	−	−
12	B	99.5	1000	−	−	−	−
13	C	99.4	1000	+	+	+	+
14	C	99.0	125	−	−	+	+
15	C	99.0	1000	−	−	−	−
16	C	99.8	1000	−	−	−	−
17	C	99.7	1000	−	−	−	−
18	C	99.6	1000	−	−	−	−

a) Componds in same category have similar structures

centration. The genotoxicity of the sample substance was evaluated with the Genox/Cytox ratio. When the ratio was 1.5 or higher in non-cytotoxic concentrations, with a dose dependent increase, the sample was judged as positive for genotoxicity (DNA-damaging activity).

SOS-inducing potency (SOSIP) was calculated as described by Quillardet et al. (1982) with minor modifications. SOSIP, defined as Genox/Cytox ratio per nmol of compound, was calculated from the data showing linearity in the dose-response curves.

Results and Discussion

Table 1 summarizes the results of the Vitotox™ test for 59 commercially available compounds previously examined with the Ames test. The results of the SOS chromotest and the *umu* test are also listed in this table for comparison.

Twenty-five Ames positive compounds gave positive results in the Vitotox™ test. Three compounds, styrene oxide, 1,2-epoxybutane and 8-hydroxyquinoline, gave negative results with this assay. Styrene oxide and 1,2-epoxybutane are reported to be positive with the SOS chromotest and the *umu* test (Quillardet and Hofnung, 1993; Reifferscheid and Heil, 1996). This discrepancy could be explained by the fact that investigated doses in the present study were limited to the low dose range because the 96-well microplates used were cauterized by the high concentrations. Reportedly, 8-hydroxyquinoline is not genotoxic in the SOS chromotest and the *umu* test (Quillardet and Hofnung, 1993; Reifferscheid and Heil, 1996). Possibly 8-hydroxyquinoline exerts its mutagenicity through an SOS-independent pathway. Five Ames positive compounds, 9-aminoacridine (9-AA), 2,4-diaminotoluene (2,4-DAT), 2,6-diaminotoluene (2,6-DAT), 1,2-dimethylhydrazine (DMH), and hydroxyurea, gave inconclusive results because they induced light emission not only in the Genox strain but also in the Cytox strain. 9-AA gave an equivocal result in the SOS chromotest and, with longer exposure, a positive result in the *umu* test (Nakamura et al., 1987). 2,4-DAT and 2,6-DAT are not genotoxic in the normal *umu* test, although their genotoxicity could be detected with a special strain NM2009 overexpressing *O*-acetyltransferase (Oda et al., 1995). As for DMH, there are conflicting results observed in the *umu* test (Reifferscheid and Heil, 1996). Overall, the results of the Vitotox™ test for the Ames positive compounds were in agreement with those of the SOS chromotest and the *umu* test.

Twenty-one Ames negative compounds gave negative results with the Vitotox™ test. Five compounds, caprolactam, d-mannitol, isophorone, thioacetamide, and urethane, gave inconclusive results, because they enhanced light emission in both the Genox and the Cytox strains.

As described above, five Ames positives and five Ames negatives were inconclusive in this study. Thus, comparisons of the results obtained in the Ames test with those obtained with the Vitotox™ test could be made for 49 compounds. The sensitivity of the Vitotox™ test for the Ames positive compounds was about 89% (25/28); negative specificity was 100% (21/21). The concordance between the Vitotox™ test and the Ames test was 94% (46/49).

We also evaluated the genotoxicity of 18 chemicals synthesized at Mitsubishi Pharma Corporation for a practical

validation of the Vitotox™ test. This assay detected 7 of 8 Ames positive compounds as genotoxic and all of the Ames negative compounds gave negative results (Table 2). The concordance between the Vitotox™ test results and the Ames test results for the 18 drug candidates was 94% (17/18). This concordance is comparable to that in the well-known, commercially available compounds and we concluded that this assay would be practical for preliminary genotoxicity screening.

We plotted the SOSIP measured with the Vitotox™ test versus the mutagenic potency calculated from the Ames test results for representative Vitotox™/Ames positive compounds. As shown in Fig. 1, the SOSIP correlated closely with the mutagenic potency. A similar correlation is observed between the SOS chromotest and the Ames test (Quillardet et al., 1982). These facts suggest that the measurement of bacterial SOS system induction is a reasonable method for predicting mutagenicity in bacteria.

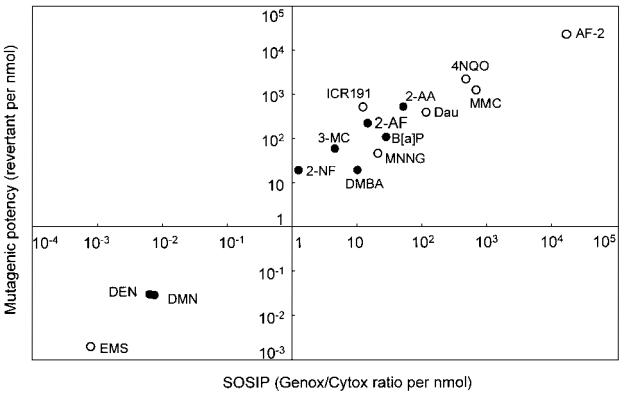

Fig. 1 Correlation between the SOSIP and mutagenic potency, calculated from the Ames test results. Open symbols, compounds tested without metabolic activation. Closed symbols, compounds tested with metabolic activation.

Table 3 shows the comparison of minimal detectable concentration and minimal quantity of sample required to detect genotoxicity with the Vitotox™ test, the SOS chromotest, the *umu* test, and the Ames test. The minimal detectable concentrations with the Vitotox™ test were lower than those in the SOS chromotest and the *umu* test (except for the *umu* test results with ICR191). The results were almost comparable with those reported by van der Lelie et al. (1997). The Vitotox™ test requires a smaller quantity of chemical than the Ames test and other 96-well formatted SOS response-based assays. This is a clear advantage when screening new drug candidates because only very small quantities of compounds are usually available in the early stage of drug discovery. Less than 10 mg is a sufficient quantity for use with the Vitotox™ test.

Unexpectedly, we obtained a high incidence of inconclusive results caused by the SOS-independent enhancement of light production (17%, 10/59). Although the detailed mechanism is unclear, there are some possible explanations for the SOS-independent enhancement of light emission. One is the enhancement of cellular metabolism by chemicals. This might occur in other SOS response-dependent assays. Another possibility is the direct effect of chemicals on the light production process. The *luxCDABE* consists of 5 structural genes. *LuxA* and *B*

Table 3 Comparison of minimal detectable concentraion/amount of chemicals in bacterial genotoxicity assays

	Minimal detectable concentration (nM)				Minimal detectable amount (ng/assay)				
	Vitotox™ (Present study)	Vitotox™ [a]	SOS[b]	*umu*[c]	Vitotox™ (Present study)	Vitotox™ [a]	Ames[d]	SOS[d] (96-well)	*umu*[d] (96-well)
2-AA	*9.8×10*	N.A.	N.A.	1.3×10^3	*1.9*	N.A.	1.3×10^2	1.8×10^2	8.9×10
2-AF	*5.5×10²*	1.1×10^3	3.7×10^5	N.A.	*1.0×10*	2.0×10	1.2×10^2	6.5×10^2	7.9×10^2
IQ	*4.5*	N.A.	N.A.	1.5×10^2	*9.0×10⁻²*	N.A.	N.A.	N.A.	N.A.
Trp-P-2	*1.8*	N.A.	N.A.	5.8×10^2	*4.7×10⁻²*	N.A.	1.4×10	N.A.	N.A.
EMS	*1.0×10⁶*	2.1×10^6	1.7×10^6	N.A.	*1.3×10⁴*	2.6×10^4	N.A.	N.A.	N.A.
MMS	*1.8×10⁴*	7.3×10^4	6.7×10^5	2.4×10^5	*2.0×10²*	8×10^2	7×10^4	N.A.	N.A.
ENNG	*3.1×10²*	N.A.	N.A.	2.7×10^3	*5.0*	N.A.	N.A.	N.A.	N.A.
MNNG	*2.7×10²*	N.A.	5.0×10^2	4.1×10^3	*4.0*	N.A.	4.2×10^2	N.A.	N.A.
B[a]P	*4.0×10²*	1.6×10^3	3.3×10^3	4.0×10^3	*1.0×10*	4.0×10	1.3×10^2	2.7×10^2	6.0×10^2
DMBA	*4.3×10²*	N.A.	2.7×10^3	3.5×10^3	*1.1×10*	N.A.	1.9×10^3	8.0×10^2	9.9×10^2
3-MC	*1.4×10³*	N.A.	6.7×10^3	2.8×10^3	*3.8×10*	N.A.	4.0×10^2	N.A.	N.A.
2-NF	*4.7×10³*	N.A.	3.3×10^5	1.5×10^5	*1.0×10²*	N.A.	N.A.	N.A.	N.A.
4NQO	1.6×10	*4.2*	2.0×10	4.2×10^2	*3.0×10⁻¹*	8.0×10^{-2}	1.9×10	5.0	1.6×10
ICR191	6.9×10^2	6.9×10^2	N.A.	*4.9×10²*	*3.1×10*	*3.1×10*	1.9×10^2	5.7×10	1.3×10^2
MMC	*1.2×10*	4.7×10	1.7×10	1.5×10^2	*3.9×10⁻¹*	1.6	N.A.	N.A.	N.A.
AF-2	*1.0×10⁻¹*	N.A.	N.A.	8.1	*1.3×10⁻³*	N.A.	2.8	N.A.	N.A.
Dau	*5.5×10*	N.A.	1.3×10^2	4.6×10^2	*3.1*	N.A.	5.6×10	1.6×10^2	3.2×10^2
NMU	*7.6×10³*	N.A.	4.0×10^4	1.2×10^5	*7.8×10*	N.A.	N.A.	N.A.	N.A.

Italics indicate the lowest value.

a) Vershaeve et al., 1999; b) Quillardet et al., 1985; c) Nakamura et al., 1987; d) McDaniels et al., 1990

genes encode subunits for luciferase. *LuxC, D* and *E* genes encode enzymes responsible for the synthesis and recycling of the aldehyde substrate for luciferase. Bacteria possessing *luxCDABE* express five proteins and produce the substrate from their own fatty acids (Meighen, 1991). The *luxCDABE* reporter system enables simple measurement of light emission from bacteria without cell disruption and without the addition of substrate. However, there is a possibility that this complicated system is affected by chemicals. In fact, the SOS-independent induction by urethane may be specific for the Vitotox™ test because this chemical did not induce a positive response in the SOS chromotest or the *umu* test (Table 1). To evaluate the genotoxicity of these compounds, an SOS response-based test with another simple reporter system such as GFP expression vector (Kostrzynska et al., 2002) or other organisms (e.g., Yeast) (Afanassiev et al., 2000; Jia et al., 2002) may be useful as alternatives.

In conclusion, the Vitotox™ test is useful for the genotoxicity screening of newly synthesized chemicals in the early stage of pharmaceutical development because it is rapid and requires only a small quantity of chemical.

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