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The SOS chromotest: a review [a,b]

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Summary

The SOS chromotest is reviewed through over 100 publications corresponding to the testing of 751 chemicals. 404 (54%) of these chemicals present a genotoxic activity detectable in the SOS chromotest. Their SOS inducing potencies span more than 8 orders of magnitude.

For 452 compounds, the results obtained in the SOS chromotest could be compared to those obtained in the Ames test. It was found that 373 (82%) of these compounds give similar responses in both tests (236 positive and 137 negative responses). Thus the discrepancies between both tests concern 79 compounds (18%). A case by case analysis shows that many of these compounds are at the same time very weak SOS inducers and very weak mutagens. Thus we think that, most of the time, the discrepancies between the two tests may be accounted for by differences in the interpretation of the results rather than by the experimental results themselves. However, there are some compounds which are clearly SOS inducers but devoid of mutagenic activity in the Ames test (such as quinoline-1-oxide) and to a larger extent, clearly mutagenic compounds which do not induce the SOS response in the SOS chromotest (such as benzidine, cyclophosphamide, acridines, ethidium bromide).

We also analyzed the correlation between SOS induction, mutagenesis and carcinogenesis according to the classification of Lewis. For 65 confirmed carcinogens (class 1), the sensitivity, i.e., the capacity to identify carcinogens, was 62% with the SOS chromotest and 77% with the Ames test. For 44 suspected carcinogens (class 2), the sensitivity was 66% with the SOS chromotest and 68% with the Ames test.

Thus, we confirmed previous observations made on 83 compounds that there is a close correlation between the results given by both bacterial tests. The capacity of the Ames test to identify carcinogens is higher than that of the SOS chromotest. However, because the number of false positive compounds was lower in the SOS chromotest, the specificity, i.e., the capacity to discriminate between carcinogens and non-carcinogens of the SOS chromotest, appeared higher than that of the Ames test. Thus, the results of the SOS chromotest and of the Ames test can complement each other.

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[a] This paper is dedicated to the memory of Olivier Huisman who participated in the early work on the SOS chromotest.

[b] The SOS chromotest results, the Ames test results and the carcinogenesis results on the chemicals discussed in this review are available in the form of a computer data base built with the program 4D for Macintosh computers. A version of this data base will be made directly accessible by ftp at ftp.pasteur.fr. Directory pub/SOSdb.

The SOS chromotest is one of the most rapid and simple short-term test for genotoxins and is easily adaptable to various conditions, so that it could be used as an early – perhaps the earliest – test in a battery.

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Introduction

Bacteria played an essential role in the origin of short-term tests designed to detect genotoxic agents. Although there is some debate on the exact predictive values for carcinogenicity of bacterial tests, it is clear that there is a strong correlation between the ability of chemicals to be genotoxic to bacteria and their mutagenic and tumor-initiating properties in mammals (Mohn,

1981; Purchase, 1982). Because bacteria grow rapidly in simple defined media, bacterial short-term tests are among the simplest, quickest and least expensive to conduct. It is largely for these reasons that they play an already important and increasing role in the screening for genotoxic agents and as a basis for regulating their use (Venitt et al., 1986; Lave and Omenn, 1986).

Since 1975, many bacterial short-term tests have been proposed (Venitt et al., 1986). All bacterial short-term tests consist of two components: the target bacterial cells and the metabolizing system. The metabolizing system is used to mimic the metabolism of the compound in mammalian cells. It is supplied exogenously, although the target bacterial cell has often its own way of metabolizing the compound tested. Thus, the compound to be tested is incubated with these components and a response is monitored. The response can be the appearance of bacterial mutants, the production of temperate bacteriophages, of colicins, the expression of genes induced by genotoxic agents, the increased killing of cells devoid of certain DNA repair systems, etc. (Hofnung and Quillardet, 1986; Venitt et al., 1986).

The Ames test, also called Salmonella/microsome assay (Ames et al., 1975; Maron and Ames, 1983) played a critical role in the spread of bacterial tests. This test measures reverse mutations from histidine auxotrophy to prototrophy in several especially constructed mutants. It is now the most extensively used as well as the bacterial short-term test validated on the largest scale in a number of laboratories (Kier et al., 1986).

Ten years ago, in 1982, we presented a bacterial assay for genotoxins: the SOS chromotest (Quillardet et al., 1982). The SOS chromotest is a colorimetric assay which measures the expression of genes induced by genotoxic agents, in *Escherichia coli*, by means of a fusion with the structural gene for β-galactosidase. The main advantages of the SOS chromotest are practical. A single strain is required. A quantitative colorimetric response is obtained within a few hours. The manipulations are very simple since only test tubes are needed. We published a validation study of the SOS chromotest with 83 compounds of various chemical classes in 1985 (Quillardet et al., 1985). In this study we found that the results obtained with the SOS chromotest were most of the time correlated with those obtained in the Ames test and we proposed the use of the SOS chromotest as a primary screening tool and as a part of a battery of short-term tests for carcinogens. Since 1982 the tester strain used in the SOS chromotest has been delivered to a large number of laboratories. Several validation studies by other laboratories have been published (Ohta et al., 1984a; Mamber et al., 1986; Brams et al., 1987; Von der Hude et al., 1988; McDaniels et al., 1990). In addition, a series of papers on the SOS chromotest issued from a number of laboratories, describing various aspects which can be examined with the test, have been published (see later in this paper).

In this paper we review the available results obtained with the SOS chromotest and we reevaluate the performances of the SOS chromotest with a larger set of data.

SOS chromotest: principle and tester strain

A genotoxic agent often makes a variety of DNA lesions. Depending on its nature and its position, in particular with respect to the replication fork, each lesion may lead to at least four types of consequences which are neither mutually exclusive nor exclusive of other consequences. (1) The lesion may be processed without mutagenic effects. This may occur by 'reversion' or by 'excision' of the lesion. (2) The lesion may provide direct miscoding. (3) The lesion may induce the SOS response which includes SOS mutagenesis. (4) The lesion may induce other specific responses such as the adaptive response to alkylating agents, the response to oxidative agents, the heat-shock response (see Hofnung and Quillardet, 1986).

The SOS response (see review by Walker, 1984; Devoret, 1992) plays a central role in the response of *Escherichia coli* to genotoxic agents because of its large spectrum of responses towards a wide variety of agents. Triggering of the system can thus be used as a general and early sign of DNA damage. Two genes play a key role: *lexA* encodes a repressor for all the genes of the system (over 15 are known), and *recA* encodes a

protein able to cleave (or to promote cleavage of) the LexA repressor upon activation by an SOS inducing signal. The exact nature of the SOS inducing signal remains mysterious. However, it is produced when DNA lesions 'perturb' or stop DNA replication.

Monitoring the triggering of the SOS system could be performed by assaying various end-points such as activation of the RecA protein, cleavage of the LexA repressor, expression of any of the SOS genes, etc. A simple way consists in monitoring the expression of an SOS gene by means of a fusion with *lacZ*, the structural gene for *E. coli* β-galactosidase.

The SOS chromotest (Quillardet et al., 1982) makes use of a *sfiA::lacZ* operon fusion; *sfiA* is one of the SOS genes. The assay consists of incubating the tester strain with increasing concentrations of the agent to be tested. After a time for protein synthesis, β-galactosidase activity is assayed by a simple colorimetric assay. A microsomal activation preparation can be included in the incubation mixture. The chemical tested may, at certain concentrations, inhibit protein synthesis, which would lead to an underestimation of β-galactosidase induction. In extreme cases this would result in false negatives. To correct for this, general protein synthesis during the incubation period can be estimated by assaying alkaline phosphatase in parallel with β-galactosidase. The assay takes only a few hours. It is quantitative, and dose–response curves present a linear region. The slope of the linear region allows unequivocal association with each compound active in induction of a single parameter, the SOS inducing potency (SOSIP), which reflects the inducing activity of the compound (Quillardet et al., 1982). For inactive compounds a 'less-than' value can be calculated.

The *E. coli* PQ37 tester strain used in the SOS chromotest carries a *sfiA::lacZ* fusion and has a deletion of the normal *lac* region, so that β-galactosidase activity is strictly dependent on *sfiA* expression. In addition, the strain is made genetically more susceptible to genotoxic agents: it is devoid of the excision repair pathway (*uvrA* mutation) so that a number of lesions are not, or are slowly, processed, and it has a mutation (*rfa*) which renders the cell envelope more permeable to a number of compounds. Constitutive synthesis of alkaline phosphatase, an enzyme independent of SOS control, serves as a control for toxicity. A detailed description of the tester strain and its construction is given in Quillardet and Hofnung (1985).

In addition to this general-purpose tester strain, several derived strains carrying mutations affecting DNA metabolism have been devised in order to provide more information on the genotoxic action of a chemical (Quillardet and Hofnung, 1988; see later in this paper).

SOS chromotest procedures

Original quantitative procedure and its improvements

Briefly, the standard procedure for the SOS chromotest consists in incubating the compound to be tested with a 10-fold diluted exponential phase culture of the tester strain. The culture is diluted either in fresh medium, or in a metabolizing mixture when activation of the compound is required. After 2 h incubation to allow for protein synthesis, this mixture is divided into two series. One series is used for the β-galactosidase assay and the other series for the alkaline phosphatase assay. The two enzyme assays can be performed in parallel. They consist in adding an appropriate buffer to disrupt cell membranes so as to liberate the enzymes into the medium. Then the mixture is brought to optimum pH for enzymatic assay. The assays are initiated by the addition of substrates specific for each enzyme. They are terminated after an appropriate time for color development by the addition of buffers which stop the enzymatic reactions. The absorbance of each assay is read against a colorimeter blank. Enzyme activities are calculated as well as the ratio of β-galactosidase units to alkaline phosphatase units (R) which reflects the induction of gene *sfiA* even when some inhibition of protein synthesis occurs. To compare results obtained in different experiments, it is convenient to normalize the ratio $R(c)$, at concentration C, by dividing it by its value $R(0)$ at concentration 0 of the compound tested. An induction factor is thus obtained. The SOSIP is the slope of the linear

region of the dose–response curves (induction factor versus concentration). The SOSIP is thus a single parameter which represents the induction factor per mass unit or per nanomole of compound tested (for details, see Quillardet and Hofnung, 1985).

We published a first procedure in 1982 (Quillardet et al., 1982), then a second detailed procedure comprising some improvements and simplifications (Quillardet and Hofnung, 1985). Since then, the general features of the procedure have remained unchanged. Only a small number of minor modifications have been proposed by other authors. These modifications are the following:

(i) Since the SOS chromotest is a colorimetric assay the reading can be obscured by strongly colored chemicals. In order to reduce the effect of the yellowish color of certain chemicals on the colorimetric assay, some authors proposed adding the compound to be tested in an undiluted or slightly diluted exponential phase culture and incubate the mixture for 1 or 2 h. Then, the mixture is diluted 10-fold, incubated for an additional 2 h for expression of protein synthesis and the enzymatic assays are performed (Ohta et al., 1984; McDaniels et al., 1990). After incubation of the mixture, a short centrifugation followed by enzyme assay on the bacterial pellet resuspended in appropriate buffer is also possible (Von der Hude et al., 1988). It is also possible to subtract the specific absorbance due to the compound to be tested (Von der Hude et al., 1988).

(ii) In order to improve the sensitivity of the enzymatic assays, slight modifications of the enzyme buffers have been proposed (Marzin et al., 1986; Mersch-Sundermann et al., 1991a). However, these authors did not agree on the optimum conditions for better results. In any case, the measure of the absorbance resulting from the enzymatic assays using a kinetic method can improve the precision of these assays (Marzin et al., 1986; Rueff et al., 1986).

(iii) The efficiency of protein synthesis may be influenced by the physiologic state and the growth rate of the bacteria. Thus some authors tried to improve the sensitivity of the test by modifying the growth conditions. Modifications for a better growth of the tester strain have been described (Mersch-Sundermann et al., 1991a).

Original spot test procedure and its improvements

We also described a simple semi-quantitative procedure, the SOS spot test, which permits a single person to test many samples on a day. In this procedure, the induction of β-galactosidase is evidenced on indicator plates containing a substrate which releases a colored dye when hydrolyzed by this enzyme. In practice, a sample of an exponential phase culture of the tester strain is added to the metabolizing mixture, when activation of the compound is required, and the mixture is plated on an appropriate minimum medium plate containing the β-galactosidase substrate. A sample of the compound to be tested is then spotted onto the center of the plate. After overnight incubation at 37°C, the presence of a blue ring around a zone of inhibition indicates that the compound can induce the *sfiA* gene (Quillardet and Hofnung, 1985).

Other spot test procedures have been described. A method described by Mamber et al. (1986) consists in plating an exponential phase culture of the tester strain on rich medium in large petri dishes which allow testing several concentrations of a number of chemicals at once. Then after 16 h incubation at 37°C, a β-galactosidase substrate is overlaid and the dishes are incubated for an additional 10–20 min for color development. The minimal active concentration, the relative size and intensity of the color reaction are taken into account for determining the relative genotoxicity of the chemical tested. Alternatively chemicals may be spotted in wells cut in the seeded dishes by using a stainless steel well-cutting device (Mamber et al., 1990). Mersch-Sundermann (1991) described a method close to that of Quillardet and Hofnung (1985) but modified the composition of the medium and the β-galactosidase substrate.

Evaluation of test results

We consider that two factors are needed to establish a positive response in the SOS chromotest: (i) an increase of 0.5 or more in the induction factor, and (ii) the observation of a dose response.

These factors have been considered differently by some laboratories although justifications are not always proposed. Thus, for Dayan et al. (1987), a substance is considered positive if the SOS induction factor is more than 1 and a dose–response relationship can be observed. For Dutka et al. (1987), values of the induction factor greater than 1.3 were considered significantly different from the negative controls. For Von der Hude et al. (1988), a result is considered positive only when the increase in the induction factor is more than 0.5 combined with an increasing β-galactosidase activity. Mersch-Sundermann et al. (1992) proposed that a chemical is 'not genotoxic' when the induction factor is lower than 1.5, 'marginal' when it is between 1.5 and 2, and 'reactive genotoxic' when it exceeds 2.

De Flora et al. (1985) defined a genotoxic chemical by its MGC (minimal genotoxic concentration), i.e., the minimal concentration inducing the production of β-galactosidase and its MIC, i.e., the minimal concentration of compound inhibiting the production of β-galactosidase.

An interesting calculation has been applied to the SOS chromotest to evaluate genotoxicity (Tenenbaum and Malaveille, 1988). In this method a genotoxic yield potency is defined by the 'yield versus lethal hit plot' of the agent tested (lethal hits) allowing direct comparison of agents of different natures (chemicals versus radiations for example). One advantage is that the abscissa depends on the biological effects (Haynes and Eckardt, 1980; Eckardt and Haynes, 1980; see later in this paper).

Although it is essentially a qualitative method, it is possible to roughly quantify the results obtained in the SOS spot test according to the relative size and intensity of the color reaction. As proposed by Mamber et al. (1986) the chemicals can be classified as: 0 = negative, +1 = equivocal, +2 = weak positive, +3 = moderate positive, +4 = strong positive.

Metabolizing mixture

We described (Quillardet and Hofnung, 1985) a metabolizing mixture for the SOS chromotest close to that described by Maron and Ames (1983). The concentration of all the components was adjusted in order to obtain convenient activation of both aflatoxin B1 and benzo[*a*]pyrene. As already noticed by others (Ames et al., 1975; Bartsch et al., 1975; Foster et al., 1980), we reported that, for certain compounds, activation may be relatively different in liquid media, as in the SOS chromotest, and on plates as in the Ames test (Quillardet et al., 1985). Thus, it appeared that modification of the metabolizing mixture could be a way to improve the performance of the SOS chromotest. However, only little work has been performed to improve the metabolizing mixture. Some authors modified the concentration of rat liver homogenate (Marzin et al., 1986; Von der Hude et al., 1988; Mersch-Sundermann et al., 1991a), but the components of the metabolizing mixture were not changed.

Procedure extensions: microsuspension methods; kit; automation

In order to facilitate the manipulation and to use a multi-channel spectrophotometer, several authors performed the assay by a microsuspension method using microtitration plaques (Rueff et al., 1986; Marzin et al., 1986; Xu and Schurr, 1990; Nair et al., 1991). The use of an automatic microplate reader allows a kinetic determination of enzymatic assays which improves the precision of these assays (Marzin et al., 1986).

As soon as 1984, the SOS chromotest was commercialized in the form of a self-contained kit (available from Orgenics Ltd). Some of the work discussed here makes use of this SOS chromotest kit (Riesenfeld et al., 1985; De Flora et al., 1985; Dayan et al., 1987; Fisch et al., 1987; Dutka et al., 1986; Kohn et al., 1988; Couture et al., 1989; Harwood et al., 1989; van Aswegen et al., 1989). However, sometimes lower performances were obtained using the kit procedure as compared to the standard test tubes procedure (Brams et al., 1987). This could be due to difficulties in the direct rehydration of dried bacteria used in the kit.

Recently, another company (Labsystems Oy, Helsinki, Finland) undertook the development of a completely automatic machine to perform the SOS chromotest (Huttunen, 1987). In this method, a self-contained interactive program controls the

test from the dilution of the stock solution of samples to be tested to the analysis and report of the final results (Janz et al., 1988, 1989). Such a machine could allow not only easy testing of a large number of compounds but also avoidance of contact between the experimentalist and the compounds to be tested. A few laboratories routinely used this automatic procedure (Tanirbergenov et al., 1988; Raabe et al., 1989; Vasilieva et al., 1990; Nylund et al., 1992).

Adaptation of the SOS chromotest to specific problems

Attempts to adapt the SOS chromotest to detect the presence of genotoxic compounds in environmental complex mixtures or biological samples have been made.

Detection of genotoxicity in urine, serum or plasma

The SOS chromotest has been used to monitor the presence of genotoxic compounds in urine samples. De Meo et al. (1988) failed to detect urinary genotoxicity caused by smoking in the presence of activating mixture whereas the sample tested showed mutagenicity in the Ames test. They tried to modify the assay in several ways (metabolizing mixture composition, assay of β-galactosidase, age of the growth media) but a strong toxicity was always observed in the SOS chromotest. Similarly, Venier et al. (1989) found that the SOS chromotest was poorly sensitive to genotoxic compounds present in urine from tobacco smokers for which a mutagenic activity was detected in the Ames test. However they found a significant SOS inducing activity, with or without metabolic activation, from a urine sample obtained from a psoriatic patient, therapeutically treated with mineral coal tar, whereas a mutagenic activity was found in the Ames test only in the presence of metabolic activation. Thus these authors concluded that the SOS chromotest could detect genotoxins in urine different from those detected in the Ames test. This conclusion is strengthened by the results of Rueff et al. (1984) who found a genotoxic activity in the SOS chromotest in concentrated samples of urine from smokers whereas these samples were mutagenic in the Ames test only after hydrolysis of urines by either β-glucuronidase or aryl sulfatase. Such a result was also noticed by Kohn et al. (1988).

The procedures reported above relied on concentrated urine samples using XAD2 resins. However, Fish et al. (1987) described a procedure to detect genotoxic activity in unconcentrated urine. Only neutralization of the samples and an additional centrifugation step are required. The method, which eliminates the toxicity associated with concentrated extracts, detected genotoxic activity in urine containing 2-aminoanthracene as well as in the same sample concentrated on XAD2 column and extracted with acetone. Such a procedure was successfully applied to detect genotoxicity in urine from oil-induced toxic shock patients. Using a similar procedure, Kohn et al. (1988) successfully used the SOS chromotest to follow the genotoxic activity in urine from cancer patients receiving antineoplastic drugs. They also tested the genotoxic activity in urine samples containing an antineoplastic drug after various extraction procedures. They found that the extraction procedures tested worked well, but that the best results were obtained using SEP-PAK C18 resin rather than XAD2.

The discrepancies between these results may be accounted for by the extraction procedure used in the tests. De Meo et al. (1988) and Venier et al. (1989) used a classical extraction procedure working well in the Ames test (i.e., concentration on an XAD2 column, then elution with acetone, evaporation and resuspension in DMSO) and the concentration factors were high: respectively 4000 and 250. Since their samples were highly toxic and since only few dilutions were tested, it is possible that in the SOS chromotest the urine samples require a lower concentration factor to yield a detectable response. Indeed, the samples used by Kohn et al. (1988) were concentrated 10-fold only. In addition, they were eluted from the column with methanol and not evaporated to dryness allowing the suppression of the resuspension step. Thus it is likely that the conditions of extraction of the urine samples required to evaluate the mutagenicity in the Ames test are quite different from those required to evaluate genotoxicity in the SOS chromotest.

Similar procedures were also used by Kohn et al. (1988) to assess genotoxicity using the SOS chromotest in serum or plasma. The assay was possible provided the plasma was first inactivated at 56°C to remove its bactericidal properties.

Detection of genotoxicity in fecal extracts

Venitt and Bosworth (1986) failed to detect the presence of genotoxic activity in 6 aqueous fecal extracts, 5 of them showing mutagenic activity in the Ames test. Nair et al. (1990) investigated the influence of dietary fat on fecal genotoxicity using the SOS chromotest. They observed that genotoxicity decreased during the low-fat period whereas fecapentaene levels increased. However, fecapentaenes are highly active in the SOS chromotest and were suspected to be responsible for the genotoxicity of fecal extracts (Nair et al., 1991). Again, the extraction procedures may be of critical importance in such studies.

Detection of genotoxicity in environmental samples

Similarly, attempts have been performed to use the SOS chromotest to detect or to evaluate genotoxic environmental contaminants.

Organic extracts from atmospheric particles in Paris were monitored during several months (Courtois et al., 1988). A genotoxic activity was generally detected but this activity disappeared in the presence of the metabolization mixture, whereas mutagenic activity was always detected using the Ames test with or without the presence of the metabolizing mixture. By contrast, Schleibinger et al. (1989) found that extracts of airborne particulate matter were genotoxic in both the Ames test and the SOS chromotest, but in the SOS chromotest these extracts revealed higher activity in the presence of metabolizing mixture.

The capacity of the SOS chromotest to detect the genotoxicity of complex environmental mixtures containing polycyclic aromatic hydrocarbons has been evaluated (Nylund et al., 1992). The assays were carried out on soxhlet extracted samples of standard reference materials. Whereas urban air particulate matter extracts were mutagenic in the Ames test, a genotoxic activity in the SOS chromotest could not be clearly evidenced with these extracts. However, diesel particulate matter extracts showed a genotoxic activity in both tests.

The SOS chromotest has also been applied for various other purposes such as the detection of beverage or food genotoxic contaminations: aflatoxin B1 in orange juice (Riesenfeld et al., 1985), or in cow milk (Frémy and Quillardet, 1985), or chlorinated butenoic acids in chlorinated drinking water (Tikkanen and Kronberg, 1990), the monitoring of natural genotoxins present in food such as flavonoids in red wine (Rueff et al., 1986, 1992), or the production of genotoxic compounds in smoked food in reaction with nitrite under acidic conditions, as they are suspected to be formed in the human stomach through an interaction between nitrite and suitable amine precursors (Ohshima et al., 1989). Other examples include detection of genotoxic pollutants in the aquatic environment (Dutka et al., 1987), influence of algae on the genotoxiciy of industrial effluents (Harwood et al., 1989) or verification of the absence of genotoxic activity of extracts from plants of medicinal importance (Vargas et al., 1990).

Thus, when the genotoxic activity of biological samples or environmental complex mixtures is evaluated, it is possible that the Ames test and the SOS chromotest account for different genotoxic activities, but it is also likely that the procedures used to prepare the samples (extraction, concentration, metabolization) have different optima in the two tests. Thus the mere application of a procedure which gives optimum results in one test to the other test is not a completely valid approach.

Use of the SOS chromotest to detect antigenotoxic compounds

There is a growing interest in the field of antimutagens, anticarcinogens and therefore antigenotoxic compounds. As mentioned by several authors, the SOS chromotest can be a powerful system to search for new antigenotoxic compounds. Generally screening of antigenotoxic compounds with the SOS chromotest consists in measuring the inhibition by the compound to be tested of the SOS induction, triggered by a known

genotoxic compounds. Thus Ohta et al. (1986) measured inhibition of ultraviolet induction by 233 compounds (food and feed additives, naturally occurring compounds, antibiotics, pesticides, inorganics and other chemicals). Two compounds, 5-fluorouracil and 5-fluorodeoxyuridine, were found to inhibit UV induction. UV-induced mutagenesis was also depressed by the addition of these compounds at non-toxic concentrations. The action of these compounds is likely to occur through inhibition of the error-prone SOS repair system. The effects of ascorbic acid, a known antimutagen, on SOS induction by *N*-methyl-*N*-nitrosoguanidine (MNNG) and nitrofurans were studied using the SOS chromotest (Vojtekova and Miertus, 1986). It was found that inhibition of MNNG induction by ascorbic acid was probably caused by the acceleration of MNNG decomposition by ascorbic acid. Inhibition of nitrofuran induction was also observed although Ascorbic acid does not react with these chemicals. This observation was confirmed by the observation that SOS induction by two nitrofurans (nitrofurazone and furazolidone) was inhibited by ascorbic acid but also sodium selenite (Gajewska et al., 1990). In addition, Sato et al. (1991) evaluated the capacity of the SOS chromotest to detect a number of known antimutagens. Their effects were evaluated on several inducing treatments (UV, 4-nitroquinoline-oxide, *N*-methyl-*N*-nitrosoguanidine or benzo[*a*]pyrene). They found an inhibitory effect of ascorbic acid, glutathione, 5-fluorouracil and 5-chlorouracil, no inhibitory effect with cobaltous chloride, sodium selenite and sodium arsenite and an increased induction with vanillin.

Use of the SOS chromotest to detect genotoxic effects of radiations

In a previous study (Quillardet and Hofnung, 1984) we showed that the SOS chromotest efficiently detected the SOS inducing effect of UV light. We found that, if, as is generally believed, pyrimidine dimers (a major UV lesion) are responsible for SOS induction, a very small number of them (if not one) per cell was enough to trigger the response. Thus the SOS chromotest is very sensitive to this type of radiation. In another study (Quillardet et al., 1989), we showed that 3 types of ionizing radiation, γ-rays, accelerated α-particles and neutron particles, could induce the *sfiA::lacZ* fusion as measured in the SOS chromotest. The sensitivity of the SOS chromotest to γ-rays was confirmed elsewhere (Kosubek et al., 1990). In addition, using heavy ions (heliun, beryllium and carbon ions), a dependence of the SOSIP on the linear energy transfer (LET) was shown (Kosubek et al., 1989). The SOSIP of radiation increases with increasing LET, to 40–60 keV/μm, reaches a maximum and then decreases at greater LET values. Both because of its sensitivity and because of its practical advantages the SOS chromotest constitutes a very efficient tool to study the genotoxic effects of radiations. It can be mentioned that, for these reasons, *E. coli* strains harboring a *sfiA::lacZ* fusion have been grown in the orbiting Biocosmos 2044 satellite in order to evaluate the effects of the space environment (Bouloc and D'Ari, 1991).

The SOS chromotest and the diagnosis of genotoxins

Diagnosis of DNA lesions

Precise information on the nature of the lesions caused by a genotoxic agent may be useful in trying to predict the consequences of its action. This may have relevance for the evaluation of genetic risks for humans from exposure to chemicals. This assumption is further supported by the fact that, for certain classes of genotoxic compounds, a quantitative correlation is observed between chemical reactivity, genotoxicity in bacteria and mutagenicity in mammalian cells (Whong and Hsieh, 1977; Yano and Isobe, 1979; Eder et al., 1980; Arnaise et al., 1986).

There are two main genetic approaches to the problem of the diagnosis of lesions. One consists in introducing mutations affecting a gene involved in the processing of a lesion (or a category of lesions) into a general-purpose tester strain, such as the SOS chromotest tester strain PQ37, and see the effects on the response. The other approach consists in monitoring the induction of genes involved in a specific response to an agent or a class of agents such as the response to

alkylating agents (*ada* gene). We, and others, have described several diagnostic strains, derived from the general tester strain PQ37, able to yield additional information on the nature of the DNA lesions caused by a genotoxic agent (see below).

Use of mutants affected in DNA metabolism

It is well known that the excision repair system, dependent on the *uvr* genes, is quite efficient in eliminating a large number of various lesions, thus lowering the corresponding SOS response. For this reason, most bacterial tests for genotoxins make use of strains deficient in excision repair. Thus the general tester strain PQ37, used in the SOS chromotest, carries a *uvrA* mutation.

From the comparison of the responses obtained in the SOS chromotest in either the *uvrA* strain PQ37 or the uvr^+ strain PQ35 (Quillardet et al., 1985) it is possible to distinguish three classes of compounds: (1) chemicals producing DNA lesions repaired by the excision repair pathway, giving a higher response in the uvr^- strain; (2) chemicals producing DNA lesions which are not recognized by this repair pathway, giving the same response in both strains; (3) interestingly, a third minor class of genotoxic agents show a uvr^+ dependent response: these agents are more potent inducers in the uvr^+ than in the uvr^- strain. Such agents include mitomycin C, a cross-linking agent (Quillardet and Hofnung, 1985). Other agents producing DNA-DNA cross-links (cisplatinum dichlorodiammine or psoralen plus UV light) are also uvr^+ dependent mutagens (Venturini and Monti-Bragadin, 1978; Bridges et al., 1979). It is possible that unrepaired cross linkage between DNA strands is lethal rather than mutagenic. The *uvrA* gene product would be required to cleave one of the damaged DNA strands generating a lesion amenable to repair (Ohnishi et al., 1987). Thus, in *uvrA* strains, SOS induction by DNA cross links could be inhibited because of their strong toxicity.

A mutation in the *ada* gene, which codes for an alkyltransferase able to dealkylate the O-6 position of guanine (O6-alkG), does not affect the SOS chromotest response to methylating agents (Quillardet et al., unpublished observations). This is compatible with the idea that O6-MeG is a direct miscoding lesion rather than a lesion blocking DNA replication and resulting in SOS induction. In contrast, a strain harboring a *tag* mutation (PQ243) is induced at lower concentrations of several alkylating agents than the general tag^+ tester strain (Costa de Oliviera et al., 1987; Quillardet et al., unpublished observations). Indeed *tag* mutants are deficient in DNA glycosylase which specifically excises 3-methyladenine from DNA (Evensen and Seeberg, 1982; Karran et al., 1982). This lesion is known to block DNA replication and to induce the SOS system (Boîteux et al., 1984).

The *oxyR* gene appears to be a positive regulator of the regulon for defense against oxidative stress. We have shown that a strain harboring a deletion of the *oxyR* gene (PQ300) is more sensitive to hydrogen peroxide and various classes of peroxides or compounds generating peroxides in vivo than the general $oxyR^+$ tester strain used in the SOS chromotest (Goerlich et al., 1989; Quillardet et al., unpublished observations). Thus, an *oxyR* deletion mutant derivative of the standard SOS chromotest strain constitutes a diagnostic strain for the detection of a subclass of oxidative genotoxins.

Certain chemicals, such as the base anologues 2-aminopurine or 5-bromouracil, are able to promote illegitimate base-pairing (mismatches) during DNA synthesis (see Ronen, 1980). When incorporated into DNA, these chemicals give rise to DNA lesions. If the mismatches are not repaired by the mismatch correction system (see Radman and Wagner, 1986), these lesions will lead to mutations. These lesions normally do not induce the SOS functions and behave as miscoding lesions (Quillardet and Hofnung, 1987). Mismatch inducing compounds, such as 2-aminopurine or 5-bromouracil, can be efficiently detected as SOS inducers in the SOS chromotest in a strain (PQ100) harboring a *dam* mutation affecting the mismatch correction system (Quillardet and Hofnung, 1987). In addition, the SOS inducing activity of an alkylating agent such as ethyl methanesulfonate (EMS) is increased in the *dam* mutant in comparison to the general dam^+ tester strain (Quillardet and Hofnung, 1987). Indeed, O6-meG, the main mutagenic lesion produced by EMS, can base-pair with T. This pair

does not by itself trigger SOS induction. However, it appears to be recognized as a mismatch and as a substrate for the mismatch repair system, yielding SOS inducing lesions. Thus, the *dam* mutant derivative of the standard SOS chromotest strain may be used as a diagnostic strain for compounds inducing DNA mismatches.

Another strain, derived from the general SOS chromotest tester strain, harboring the *uvrA* mutation and a temperature conditional mutation in the *dnaC* gene involved in initiation of DNA replication, has been constructed (Salles et al., 1987). In this strain, at non-permissive temperature the SOS system cannot be induced by typical SOS genotoxins, such as UV light, since DNA replication is blocked. However, treating this strain with an agent producing single-strand breaks restores induction of the SOS system (Salles et al., 1987). Thus, by this method it is possible to know if a genotoxic compound is able to create single-strand breaks in DNA.

Induction of genes responding to specific DNA damage

Another approach to the genetic diagnosis of lesions consists in monitoring the induction of genes belonging to networks responding to specific DNA damages. This can be done conveniently by using gene fusion techniques. It offers the possibility of having direct diagnostic strains for chemicals inducing these networks. For example, genetic fusions between the β-galactosidase gene and genes involved in the adaptive response to alkylating agents have been constructed and could be used for the diagnosis of these agents (Nakabeppu et al., 1984; Otsuka et al., 1985). This approach could be extended to other DNA damage inducible genes.

Activation batteries

A large number of chemicals are metabolized by bacterial cells (see Callen, 1978) through various pathways. A genetic approach to the study of these pathways is powerful. Indeed, mutants affected in such pathways can be very useful in classifying chemicals, and could be at the origin of 'activation batteries'. Until now, few mutants of this type have been studied in a systematic way. A good example is the widely used nitroreductase deficient strains. Microorganisms possess a number of nitroreductases which can activate compounds such as nitrofurans. Nitroreductase deficient mutant mutants have been isolated as nitrofuran resistant strains starting from standard strains used in the Ames test (Rosenkranz and Speck, 1976) or the SOS chromotest (Quillardet et al., 1986). The genotoxic activities of nitrofurans measured in these nitroreductase deficient mutants were always lower than in parental nitroreductase proficient strains.

Thus, bacterial mutants affected in the chemical metabolism would allow the classification of chemicals. In addition, such mutants lacking bacterial enzymes would be particularly useful when the role of mammalian metabolism in activating specific compounds is to be assessed. With adequate mutants, the active metabolites would only be due to the mammalian enzymes. The species reactive to DNA produced in bacteria may be different from the ones in higher cells (Callen, 1978). It seems important to know these bacterial metabolic pathways to avoid possible misinterpretations of test results.

Other assays involving genetic fusions

In addition to the SOS chromotest, other tests based on measuring the induction of a genetic fusion between one of the SOS genes and *lacZ*, the structural gene of β-galactosidase, have been proposed to detect genotoxic compounds.

β-Galactosidase (lacZ) fusions

The biochemical prophage induction assay, B.I.A. (Elesperu and White, 1983), monitors the induction of a genetic fusion between the C1 repressor of phage lambda and the *lacZ* gene in a strain lysogenic for a derivative of phage lambda. As we have already discussed (Quillardet et al., 1982, 1985), since the β-galactosidase assay reflects lambda repressor cleavage, the B.I.A. has an inherent limit in sensitivity. Indeed, it is now known that the lambda repressor is relatively resistant to cleavage by the RecA protein, activated by DNA damage, in comparison with the LexA repressor, the repressor of all bacterial

SOS genes. This observation was confirmed by the fact that compounds active in both the B.I.A. and the SOS chromotest were active at 10–100-fold lower concentrations in the SOS chromotest (Mamber et al., 1986). Some compounds inducing the SOS response in the SOS chromotest could not be detected using the B.I.A.

Other tests, based on the principle of the SOS chromotest, have been devised. They measure the induction of an SOS gene (*umuC* or *recA*) under LexA control by means of a fusion with the *lacZ* gene. They are the Umu test (Oda et al., 1985) and the RecA test (Nunoshiba and Nishioka, 1991). Since, in all these assays, the initial event which triggers β-galactosidase induction is the same, i.e., the cleavage of the LexA repressor, one would generally expect similar results in each of these assays. However, one can imagine that different affinities of binding of the LexA protein to the regulatory regions of the various SOS genes could produce differences in the induction of these genes.

Few studies comparing the sensitivity in the induction of the different SOS genes have been performed. Only a study by Ysern et al. (1990) compares the induction of *sfiA*, *umuC* and *recA* genes fused to *lacZ*, by mitomycin C, *N*-methyl-*N*-nitrosoguanidine, nalidixic acid and 3 fluoroquinolones, in a similar genetic background. They found that the 3 genetic fusions were induced at similar concentrations of compounds and that the levels of their expression were very close. Similar observations were made when the induction was triggered by chromium compounds (Llagostera et al., 1986). However, it has been observed (Llagostera et al., 1987) that, in the absence of metabolic activation, the inducing effect of quercetin was higher for *sfiA* than for *recA* and *umuC*. However, in the presence of metabolic activation, the induction of *sfiA* gene was decreased whereas the opposite effect was observed for *umuC* and *recA*.

It can also be mentioned that beside the SOS gene which is monitored, the SOS chromotest differs from the Umu-test and the RecA-test by some other points which may influence the results. First, the SOS chromotest makes use of an *E. coli* strain whereas the other tests make use of *Salmonella typhimurium* strains. Although these strains are enterobacteria, some differences in the metabolism of chemicals may occur as well as some differences in the processing of the DNA lesions. Furthermore, whereas in the SOS chromotest the *sfiA::lacZ* fusion is integrated in the chromosome, in the other tests the *umuC::lacZ* or the *recA::lacZ* fusions are carried by a multicopy plasmid. In addition, in the SOS chromotest, measuring alkaline phosphatase is performed as a measure for general toxicity. The strains used in the Umu-test and the RecA-test are not constitutive for alkaline phosphatase so that such a measurement cannot be performed. It is replaced by measuring cell density (absorbance at 600 nm), rendering the procedures dependent on various factors such as cell shape.

To our knowledge only one study which compares the results of the SOS chromotest (the SOS chromotest kit was used) to those of the Umu-test was performed with several compounds (McDaniels et al., 1990). They found that both methods produced nearly identical rankings of sensitivity. However, these authors noticed that whereas the SOS chromotest was very sensitive for direct acting genotoxins, i.e., which do not require metabolic activation, the Umu-test was better adapted to genotoxins requiring metabolic activation to be active. This may be valid only for the SOS chromotest kit. Other explanations are possible, such as a different behavior of the bacterial strains (*E. coli* versus *S. typhimurium*) in the presence of the metabolizing mixture.

Nevertheless, for the purpose of mass screening of genotoxic agents, the differences observed between the test systems based on the colorimetric measurement of one of the SOS genes are probably not critical.

Multitest

The *E. coli* multitest is an ingenious method to evaluate the effects of genotoxic agents by monitoring three effects: SOS induction (I effect), mutagenesis (M effect) and recombination (R effect). A set of two strains (MT103 and MT119) allows a quantitative evaluation of these three effects in the same genetic background by counting Gal^+ and Lac^+ colonies (Toman et al., 1985; Tenenbaum et al., 1988; Pionneau et al., 1992).

The I and M effects are detected thanks to a genetic fusion which results in the expression of the bacterial *gal* genes being controlled by the immunity region of phage lambda. The test makes it possible to distinguish between proteolytic cleavage of the CI repressor (I effect, strain MT119) and its inactivation by mutations (M effect, strain MT103). As discussed above in the case of the B.I.A. test, measuring of the I effect is inherently less sensitive than assay of the induction of a bacterial SOS gene. The R effect is measured through the appearance of Lac^+ recombinants between two mutated copies of the *lacZ* gene.

Quantification of the results was perfomed by two different approaches. The first one is the classical dose related potency which is derived from dose–response curves. The frequency of altered bacteria among survivors or the yield of altered cells per cells initially treated is expressed as a function of the dose of chemical. The second one consists in expressing the frequency of altered bacteria in terms of the number of lethal hits, that is a parameter which reflects a biological effect of the agent tested (Eckardt and Haynes, 1980; Haynes and Eckardt, 1980). This approach has the advantage of permitting a comparison of the effects of agents whose physical exposure doses otherwise would be incommensurable.

Tenenbaum and Malaveille (1988) quantified the genotoxic effects of 11 monofunctional alkylating agents in the multitest, SOS chromotest and Ames test, using one or the other of these calculation methods. They found that mutagenesis measured either in the Ames test or in the multitest was correlated with SOS induction measured either in the SOS chromotest or in the multitest or with recombination measured in the multitest when the genotoxic events were quantified by the dose related potency parameter. However, when the genotoxic events were quantified by the yield potency parameter, a lack of correlation was found between the mutagenic yield potency and the SOS inducing yield potency. The SOS inducing yield potency was only correlated with the recombinogenic yield potency. These observations were valid when the three genotoxic events were measured with the multitest as well as when they were measured by the Ames test (mutagenesis) or the SOS chromotest (SOS induction). The authors concluded that mutagenesis in the Ames test was not significantly correlated with SOS induction in the SOS chromotest and therefore the SOS chromotest should be considered a complement rather than an alternative to the Ames test.

To our knowledge, these comparisons were only performed on a set of monofunctional alkylating agents and cannot be generalized, at present, to other genotoxic agents. Indeed, a characteristic of monofunctional alkylating agents is the production of O6-alkG, a direct miscoding lesion responsible for mutagenesis but which does not block DNA replication (at least for short alkyl chains) and therefore does not result in SOS induction. Thus these agents could constitute a particular class for which mutagenesis and SOS induction would be uncoupled.

Analysis of the data on the SOS chromotest

We were aware of more than 100 publications on the SOS chromotest from about 40 different laboratories. From these different publications we could draw out data for 751 compounds examined in the SOS chromotest. Series of similar compounds have been grouped in tables. Because often results were not presented in a quantitative way in the original papers, we decided to report only qualitative data in the tables in the form of positive or negative responses. This decision results in a loss of information for a number of compounds for which quantitative data are available (see for example Kier, 1988), but allows a much larger number of tested compounds to be considered. When available, data for mutagenicity in the Ames test and data for carcinogenicity were also reported (see legends of tables). 404 of these compounds presented a genotoxic activity in the SOS chromotest. Their SOSIP varied from about 10^{-3} to 10^6.

Reproducibility of the SOS chromotest

From 103 compounds which were tested by 2 or more different laboratories, conflicting results between laboratories were reported for 19 compounds (Table 1). On this basis the test viability is

TABLE 1

COMPOUNDS TESTED IN THE SOS CHROMOTEST BY MORE THAN 2 LABORATORIES AND FOR WHICH CONFLICTING RESULTS ARE REPORTED

Chemical name	SOS induction
2-Acetylaminofluorene	+ (1,3); +/− (4)
Acrolein	− (1); + (78)
9-Aminoacridine	− (5,3,1); +/− (4)
2-Aminofluorene	+ (1,2,3,12,67); − (4)
Ampicillin	− (53); +/− (4)
Benzo[*e*]pyrene	− (2); + (81)
5-Bromo-2′-deoxyuridine	+ (45) (24); − (1)
Chromium acetate (CrIII)	− (16); + (53)
Crotonaldehyde	− (1); + (78)
1,3-Dichloropropanol	− (1); + (77)
Ethidium bromide	− (3); +/− (4)
5-Fluorouracil	+ (45); − (4)
Methotrexate	+ (45); − (4)
2-Nitrofluorene	+ (2,3,4,9,65); − (1)
4-Nitro-*o*-phenylene-diamine	+ (67); − (3)
N-Nitroso-diethylamine	+ (2); − (1)
N-Nitroso-dimethylamine	+ (2); − (1)
Penicillinic acid	+ (8); − (7)
Pyrene	+ (2); − (81)

Data for SOS induction are (−) negative, (+) positive, (+/−) equivocal test results. Each result is followed by its reference number: (1) Von der Hude et al., 1988; (2) Quillardet et al., 1985; (3) Ohta et al., 1984a; (4) Mamber et al., 1986; (5) Podger and Hall, 1984; (6) Fish et al., 1987; (7) Krivobok et al., 1987; (8) Auffray and Boutibonnes, 1986; (9) Marzin et al., 1986; (10) Thybaud-Lambay et al., 1986; (11) Riesenfeld et al., 1985; (12) De Flora et al., 1985; (13) Rueff et al., 1986; (14) Quillardet et al., 1986; (15) Koukalova et al., 1986; (16) Llagostera et al., 1986; (17) Rempola et al., 1986; (18) Bosworth and Venitt, 1986; (19) Venitt and Bosworth, 1986; (20) Miertus et al., 1986; (21) Vojtekova and Miertus, 1986; (22) Sturdikova et al., 1986; (23) Dayan et al., 1987; (24) Quillardet and Hofnung, 1987; (25) Basler et al., 1987; (26) Miertus et al., 1987; (27) Sturdik et al., 1986; (28) Ohta et al., 1984b; (29) Olivier and Marzin, 1987; (30) Llagostera et al., 1987; (31) Bosworth et al., 1987; (32) Potenberg et al., 1988; (33) Lecointe, 1984; (34) Arnaise et al., 1986; (35) Von der Hude et al., 1986; (36) Scheutwinkel et al., 1986; (37) Venitt and Crofton-Sleigh, 1987; (38) Van Sluys et al., 1986; (39) Tudek et al., 1988; (40) Quillardet et al., 1988; (41) Podger and Hall, 1985; (42) De Meo et al., 1988; (43) Kohn et al., 1988; (44) Eder et al., 1989a; (45) Chen et al., 1988; (46) Dale et al., 1989; (47) Eder et al., 1989b; (48) Mersch-Sundermann et al., 1989a; (49) Mamber et al., 1987; (50) Janz et al., 1988; (51) Mersch-Sundermann et al., 1989b; (52) Tikkanen and Kronberg, 1990; (53) Venier et al., 1989; (54) Ysern et al., 1990; (55) Mamber et al., 1990; (56) Von Poser et al., 1990; (57) Valsa et al., 1990; (58) Vasilieva et al., 1990; (59) Von der Hude et al., 1990; (60) Couture et al., 1990; (61) Eder et al., 1989c; (62) Gajewska et al., 1990; (63) Goerlich et al., 1989; (64) Xu and Schurr, 1990; (65) Schleibinger et al., 1989; (66) Van Aswegen et al., 1990; (67) McDaniels et al., 1990; (68) Glatt et al., 1991; (69) Henschler et al., 1991; (70) Widdick and Edwards, 1991; (71) Mersch-Sundermann et al., 1991a; (72) Moreno et al., 1991; (73) Nair et al., 1991; (74) Eder et al., 1991a; (75) Mersch-Sundermann et al., 1991b; (76) Eder et al., 1991b; (77) Hahn et al., 1991; (78) Eder et al., 1990; (79) Drobnik et al., 1987; (80) Blakeborough et al., 1989; (81) Mersch-Sundermann et al., 1992; (82) Pommery et al., 1989; (83) Masek and Sedliakova, 1992.

82%. In fact, when the conflicting results are analyzed case by case (see below), it appears that the viability of the test may be much higher.

Out of these 103 compounds, 26 were examined in the SOS chromotest by no less than 4 different laboratories independently. In this series, a concordant outcome was found for 23 compounds (21 positive and 2 negative responses). Thus the test viability reaches 88%. The 3 conflicting results were obtained for: 2-aminofluorene and 2-nitrofluorene for which 1 negative response was reported out of 5 positive responses and 9-aminoacridine for which 3 negative responses and one equivocal response (±) were reported. It is likely from these data that the 3 compounds were not adequately tested in the laboratories which report a response different from the majority.

Again, for 2-acetylaminofluorene, which was tested independently in 3 laboratories, a positive response was found in 2 different laboratories but it was reported as equivocal by another laboratory. For ampicillin and ethidium bromide one negative response and one equivocal response were reported. Each equivocal (±) response was obtained in the same laboratory which used the SOS spot test.

For most of the other compounds, when a positive response was reported, this response was weak and at the limit of detection of the test. In such a case, the discrepancy of the responses may not be due to a difference in the result itself, but rather to a difference in the interpretation of the result. Thus, one negative and one weak positive response were reported for benzo[*e*]pyrene, 5-bromo-2′-deoxyuridine, chromium acetate, 1,2-dichloropropanol, 5-fluorouracil, methotrexate, 4-

TABLE 2

COMPOUN

Chemical na
N-Acetoxy-
2-Acetylami
2-Acetyl-7-n
Acridine (IC
Acridine (IC
Acridine or
Adriamycin
Aflatoxicol
Aflatoxin B
Aflatoxin B
Aflatoxin G
Aflatoxin M
Allyl-metha
2-Aminoant
2-Aminoflu
2-Amino-5-n
Anthanthre
Azaserine
Aziridino-2,
Baldrinal
Benz[*a*]anth
anti-Benzo[
syn-Benzo[
anti-Benzo[
syn-Benzo[
Benzo[*b*]flu
Benzo[*ghi*]f
Benzo[*j*]flu
Benzo[*ghi*]p
anti-Benzo[
syn-Benzo[
Benzo[*a*]py
anti-Benzo[
syn-Benzopy
Bleomycin
Boldine
2-Bromoacr
tert.-Butylhy
2-Butyl-met
Captafol
Captan
Carboplatin
2-Chloroacr
(*Z*)-2-Chlor
(*E*)-2-Chlor
(*Z*)-2-Chlor
2-Chloro-3,3
2-Chloroeth
3-Chloro-3-n
Chromium c
Chrysene
anti-Chrysen
syn-Chrysen
Ciprofloxaci

TABLE 2

COMPOUNDS GIVING A POSITIVE RESPONSE IN BOTH THE SOS CHROMOTEST AND THE AMES TEST

Chemical name	SOS induction	Mutagenesis
N-Acetoxy-2-acetylaminofluorene	+ (2)	+ (2,a)
2-Acetylaminofluorene	+ (1,3); + / − (4)	+ (a,1)
2-Acetyl-7-methoxynaphthofuran	+ (34)	+ (34)
Acridine (ICR170)	+ (1,3)	+ (a,1)
Acridine (ICR191)	+ (1,3,41,67)	+ (a,1,67)
Acridine orange	+ (1,3,4,71)	+ (a,1)
Adriamycin	+ (4,43)	+ (a,b)
Aflatoxicol	+ (7,8)	+ (8)
Aflatoxin B1	+ (2,4,6,7,8,9,10,11...)	+ (2,a,b)
Aflatoxin B2	+ (2,7)	+ (2,b); T (a)
Aflatoxin G1	+ (2,4,7)	+ (2,b); T (a)
Aflatoxin M1	+ (2)	+ (a,2,b)
Allyl-methanesulfonate	+ (44,47)	+ (44,47)
2-Aminoanthracene	+ (1,3,4,6,12,50,53...)	+ (a,1)
2-Aminofluorene	+ (1,2,3,12,67); − (4)	+ (a,1,4,67)
2-Amino-5-nitrothiazol	+ (1)	+ (1)
Anthanthrene	+ (81)	+ (a,81)
Azaserine	+ (2,3,4)	+ (2,a)
Aziridino-2,4-dinitrobenzamide (5) (CB1954)	+ (37)	+ (37)
Baldrinal	+ (1,35)	+ (1,35)
Benz[*a*]anthracene	+ (1,81)	+ (a,1,81)
anti-Benzo[*g*]chrysene-11,12-diol-13,14-oxide	+ (68)	+ (68)
syn-Benzo[*g*]chrysene-11,12-diol-13,14-oxide	+ (68)	+ (68)
anti-Benzo[*c*]chrysene-9,10-diol-11,12-oxide	+ (68)	+ (68)
syn-Benzo[*c*]chrysene-9,10-diol-11,12-oxide	+ (68)	+ (68)
Benzo[*b*]fluoranthene	+ (81)	+ (81)
Benzo[*ghi*]fluoranthene	+ (81)	+ (81)
Benzo[*j*]fluoranthene	+ (81)	+ (81)
Benzo[*ghi*]perylene	+ (81)	T (a); + (81)
anti-Benzo[*c*]phenanthrene-3,4-diol-1,2-oxide	+ (68)	+ (68)
syn-Benzo[*c*]phenanthrene-3,4-diol-1,2-oxide	+ (68)	+ (68)
Benzo[*a*]pyrene	+ (1,2,4,9,17,25,32,42...)	+ (a,1)
anti-Benzo[*a*]pyrene-7,8-diol-9,10-oxide	+ (68)	+ (a,68)
syn-Benzopyrene-7,8-diol-9,10-oxide	+ (68)	+ (a,68)
Bleomycin	+ (2,3,4,9,43,49)	+ (b)
Boldine	+ (72)	+ (72)
2-Bromoacrolein	+ (78)	+ (78)
tert.-Butylhydroperoxide	+ (1,61)	+ (1)
2-Butyl-methanesulfonate	+ (44,47)	+ (44,47)
Captafol	+ (3,28,64)	+ (64); T (a)
Captan	+ (3,28,64)	+ (64,a)
Carboplatin	+ (79)	+ (79)
2-Chloroacrolein	+ (78)	+ (78)
(*Z*)-2-Chloro-3-(dichloromethyl)-4-hydroxybut-2-enoic acid	+ (52)	+ (52)
(*E*)-2-Chloro-3-(dichloromethyl)-4-oxobutenoic acid	+ (52)	+ (52)
(*Z*)-2-Chloro-3-(dichloromethyl)-4-oxobutenoic acid	+ (52)	+ (52)
2-Chloro-3,3-dimethylacrolein	+ (78)	+ (78)
2-Chloroethyl-methanesulfonate	+ (44,47)	+ (44,47)
3-Chloro-3-methylacrolein	+ (78)	+ (78)
Chromium oxide (CrVI)	+ (16)	+ (b)
Chrysene	+ (3,81)	+ (a,81)
anti-Chrysene-1,2-diol-3,4-oxide	+ (68)	+ (68)
syn-Chrysene-1,2-diol-3,4-oxide	+ (68)	+ (68)
Ciprofloxacin	+ (54)	+ (54)

TABLE 2 (continued)

Chemical name	SOS induction	Mutagenesis
Cisplatin	+ (4,18,19,31,43,79)	+ (b)
Cumene hydroperoxide	+ (1,61)	+ (1)
Cyclohexyl-methanesulfonate	+ (44,47)	+ (44,47)
Dacarbazine	+ (43)	+ (b)
Daunomycin	+ (2,3,4,22,49,67,71)	+ (a,2,4,67)
Dibenz[*a*,*c*]anthracene	+ (81)	+ (a,81)
Dibenz[*a*,*h*]anthracene	+ (81)	+ (a,81)
Dibenzo[*a*,*h*]pyrene	+ (81)	+ (81)
Dibenzo[*a*,*i*]pyrene	+ (81)	+ (a,81)
1,2-Dibromo-3-chloropropane	+ (3,28)	+ (a,b)
1,3-Dichloropropene	+ (1)	+ (a,b,1)
2,3-Dichloropropene	+ (1)	+ (1)
1,3-Dichlor-2-propyl-methanesulfonate	+ (44,47)	+ (44,47)
1,2:3,4-Diepoxybutane	+ (59)	+ (59)
1,2,3,4-Diepoxybutane	+ (1)	+ (a,1)
1,2:5,6-Diepoxyhexane	+ (59)	+ (59)
1,2:7,8-Diepoxyoctane	+ (59)	+ (59)
Diethyl sulfate	+ (1,2)	+ (1,2,a,b)
2,3-Dihydro-4*H*-pyran-5-carbaldehyde	+ (78)	+ (78)
Dihydrovaltrate	+ (1,35)	+ (1,35)
9,10-Dimethyl-anthracene	+ (2)	+ (2)
7,12-Dimethylbenz[*a*]anthracene	+ (1,2,3,4,9,67,81)	+ (a,1,2,67,81)
3,3-Dimethyl-2-butyl-methanesulfonate	+ (44,47)	+ (44,47)
9,10-Dimethyl carbamoyl chloride	+ (2)	+ (2,a,b)
N,*N*-Dimethyl-(2-chloro-ethyl)-amine	+ (1)	+ (1)
3,3-Dimethyl-propyl-methanesulfate	+ (44,47)	+ (44,47)
Dimethyl sulfate	+ (2)	+ (2,b); T (a)
2,7-Dinitrofluorenone	+ (75)	+ (75)
2,7-Dinitro-9-fluorene	+ (65,75)	+ (65,75)
2,7-Dinitro-9-fluorenone-4-carboxamide	+ (58)	+ (58)
1,5-Dinitronaphthalene	+ (75)	+ (75)
2,7-Dinitrophenanthrenequinone	+ (58)	+ (58)
1,3-Dinitropyrene	+ (75)	+ (75)
1,6-Dinitropyrene	+ (75)	+ (75)
1,8-Dinitropyrene	+ (75)	+ (75)
Enoxacin	+ (54)	+ (54)
Epibromohydrin	+ (59)	+ (59)
Epichlorohydrin	+ (1,2,59,71,77)	+ (a,b,1,2,59)
Epifluorohydrin	+ (59)	+ (59)
1,2-Epoxy-3-butene	+ (59)	+ (59)
2,3-Epoxy-1-propanol	+ (59)	+ (59)
1,4-bis-(2,3-Epoxypropoxy)-butane	+ (59)	+ (59)
1,2-bis-(2,3-Epoxypropoxy)-ethane	+ (59)	+ (59)
2,3-Epoxypropyl-acrylic acid ester	+ (59)	+ (59)
2,3-Epoxypropyl-1-allylether	+ (59)	+ (59)
2,3-Epoxypropyl-*n*-butylether	+ (59)	+ (59)
2,3-Epoxypropyl-*tert.*-butylether	+ (59)	+ (59)
2,3-Epoxypropyl-4-*tert.*-butylphenylether	+ (59)	+ (59)
2,3-Epoxypropyl-4-chlorophenylether	+ (59)	+ (59)
2,3-Epoxypropyl-isopropylether	+ (59)	+ (59)
2,3-Epoxypropyl-methacrylic acid ester	+ (59)	+ (59)
2,3-Epoxypropyl-4-methoxyphenylether	+ (59)	+ (59)
2,3-Epoxypropyl-methylether	+ (59)	+ (59)
2,3-Epoxypropyl-phenylether	+ (59)	+ (59)
N-(2,3-Epoxypropyl)-phthalamide	+ (59)	+ (59)

TABLE 2 (continued)

Chemical name	SOS induction	Mutagenesis
1,2-Epoxy-3,3,3-trichloropropane	+ (59)	+ (59)
Ethylenedibromide	+ (1,2,3,28)	+ (b,1,2); T (a)
Ethyl methanesulfonate	+ (1,2,3,4,44,47)	+ (a,1,2)
N-Ethyl-*N'*-nitro-*N*-nitrosoguanidine	+ (1,3)	+ (1); T (a)
Ferbam	+ (64)	+ (64)
Fluoranthene	+ (81)	+ (81)
Folpet	+ (64)	+ (64,a)
Formaldehyde	+ (1)	+ (b; 1); − (1)
Furazolidone	+ (3,62)	+ (62)
Furylfuramide	+ (3,26,27)	+ (a)
Glycidyl-trimethylammoniumchloride	+ (59)	+ (59)
Glyoxal	+ (1)	+ (1)
Homobaldrinal	+ (1,35)	+ (1,35)
Hydrazine	+ (2)	+ (2,b)
4-Hydroxyamino-quinoline-1-oxide	+ (3)	+ (a)
Hydroxyurea	+ (1,2,45,53)	+ (1,2)
Indeno[1,2,3-*cd*]-pyrene	+ (81)	+ (81)
8-Methoxy-2-nitro-anthrafuran (R7707)	+ (40)	+ (40)
2-Methylanthracene	+ (81)	+ (81)
9-Methylanthracene	+ (81)	+ (81)
Methylazoxymethanol-acetate	+ (2)	+ (2); T (a)
Methyl-butyl-methanesulfonate	+ (44,47)	+ (44,47)
3-Methylcholanthrene	+ (1,2,3,9,67,71,81)	+ (a,1,2,67)
3-Methyl-glyoxal	+ (1)	+ (1)
Methyl methanesulfonate	+ (1,2,4,39,44,47...)	+ (a,1,2)
N-Methyl-*N'*-nitro-*N*-nitrosoguanidine	+ (1,2,3,4,20,21,39...)	+ (a,b,1,2)
Methyl-propyl-methanesulfonate	+ (44,47)	+ (44,47)
Methyl vinyl ketone	+ (76,78)	+ (78)
Metronidazole	+ (4,23)	+ (a,b,23)
Misonidazole	+ (46,70)	+ (a)
(*trans-trans*)-Muconaldehyde	+ (69)	+ (69)
Nalidixic acid	+ (4,45,54,83)	+ (54)
1-Naphthylamine	+ (2)	+ (a,b)
2-Naphthylamine	+ (2,3)	+ (2,a,b)
Neocarzinostatin	+ (2)	+ (2)
Nifuroxazide	+ (23)	+ (23)
2-Nitroanthra-furan: 2 isomers [1,2-*b*] and [2,1-*b*] (R7686, R7688)	+ (40)	+ (40)
2-Nitrobenzofuran: 4 derivatives	+ (34)	+ (34)
o-Nitrocinnamaldehyde	+ (74,78)	+ (74,78)
p-Nitrocinnamaldehyde	+ (74,78)	+ (74,78)
m-Nitrocinnamic acid	+ (74)	+ (74)
p-Nitrocinnamic acid	+ (74,78)	+ (74,78)
3-Nitrofluoranthene	+ (65,75)	+ (65,75)
2-Nitrofluorene	+ (2,3,4,9,65); − (1)	+ (1,2,4,75)
3-Nitro-9-fluorenone	+ (75)	+ (75)
7-Nitro-9-fluorenone-4-carboxylic acid	+ (58)	+ (58)
Nitrofurantoin	+ (14,15,26,27)	+ (a)
Nitrofurazone	+ (14,62)	+ (a,62)
5-Nitro-2-furfural	+ (74)	+ (74)
5-Nitro-2-furylacrolein	+ (74)	+ (74)
Nitrogen mustard	+ (2)	+ (2,a,b)
2-Nitro-7-methoxynaphthofuran (R7000)	+ (2,34)	+ (2,34)
2-Nitro-7-methoxynaphthofuran (R7000): open analogue	+ (34)	+ (34)
2-Nitronaphthofuran: 14 derivatives	+ (34)	+ (34)
2-Nitrophenanthrenequinone	+ (58)	+ (58)

TABLE 2 (continued)

Chemical name	SOS induction	Mutagenesis
4-Nitro-*o*-phenylene-diamine	+ (67); − (3)	+ (a,67)
1-Nitropyrene	+ (3,65,75)	+ (65,75)
8-Nitro-pyreno-furan: isomers [1,2-*b*] and [2,1-*b*]	+ (14)	+ (14)
5-Nitroquinoline	+ (75)	+ (a,75)
4-Nitroquinoline-*N*-oxide	+ (1,2,4,6,9,12,19,25...)	+ (a,1,2)
N-Nitroso-dibutylamine	+ (2)	+ (2,a)
N-Nitroso-diethylamine	+ (2); − (1)	− (1); + (a,1,2)
N-Nitroso-dimethylamine	+ (2); − (1)	− (1); + (a,1,2)
N-Nitroso-dipropylamine	+ (2)	+ (a,2)
N-Nitroso-ethylurea	+ (1,2,3)	+ (a,1,2)
N-Nitroso-methylurea	+ (1,2,3,4,83)	+ (a,1,2)
N-Nitroso-morpholine	+ (2)	+ (2); T (a)
N-Nitroso-piperidine	+ (2)	+ (2,a)
2-Nitro-tetrahydronaphthofuran: 6 derivatives	+ (34)	+ (34)
Nitrovin	+ (3,21,26,27)	+ (a)
Ofloxacin	+ (54)	+ (54)
Olaquindox	+ (1)	+ (1)
3-Pentyl-methanesulfonate	+ (44,47)	+ (44,47)
anti-Phenanthrene-1,2-diol-3,4-oxide	+ (68)	+ (68)
syn-Phenanthrene-1,2-diol-3,4-oxide	+ (68)	+ (68)
1-Phenyl-2-butyl-methanesulfonate	+ (44,47)	+ (44,47)
2-Phenylethyl-methanesulfonate	+ (44,47)	+ (44,47)
2-Phenyl-propyl-methanesulfonate	+ (44,47)	+ (44,47)
Phosmet	+ (64)	+ (64)
β-Pinane oxide	+ (59)	+ (59)
Potassium chromate (CrVI)	+ (6,16,29,53)	+ (a,b)
Potassium dichromate	+ (53)	+ (a,b)
Proflavin sulfate	+ (3)	+ (a)
1,3-Propane sultone	+ (2)	+ (2,a)
β-Propiolactone	+ (2,9)	+ (2,a)
Propyleneimine	+ (2)	+ (2,a)
Propyl-methanesulfonate	+ (44,47)	+ (44,47)
2-Propylmethanesulfonate	+ (44,47)	+ (44,47)
Pyreno-furan: 2 isomers [1,2-*b*] and [2,1-*b*]	+ (14)	+ (14)
Quercetin	+ (3,13,23,30)	+ (a,23)
Quinoxaline-1,4-dioxide	+ (1)	+ (1)
Quinoxaline-1-oxide	+ (1)	+ (1)
Reductone	+ (57)	+ (57)
S18506 (Nitrofuran)	+ (23)	+ (23)
Sodium dichromate (CrVI)	+ (12)	+ (a,b)
Sterigmatocystin	+ (7)	+ (a)
Styrene oxide	+ (59)	+ (59)
2,4,5,7-Tetranitro-9-fluorenone	+ (75)	+ (75)
2,4,7-Trinitro-9-fluorene	+ (67,71,75)	+ (67,75)
2,4,7-Trinitrophenanthrenequinone	+ (58)	+ (58)
Triphenylene	+ (81)	+ (81)
Valtrate/isovaltrate	+ (1,35)	+ (1,35)
Vitamin K1	+ (80)	+ (80)

For SOS induction see legend of Table 1.

Data for mutagenicity in the *Salmonella typhimurium* assay (Ames test) are (−) negative, (+) positive, ? and T equivocal test results. Each result is followed by its reference number: (a) a report of the U.S. Environmental Protection Agency Gene-Tox Program (1986); (b) McCann et al., 1975b; (c) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Genetic and Related Effects: an Updating of Selected IARC Monographs from Volumes 1–42; (1–83) results given by the authors who performed the SOS chromotest on the same compounds (see legend of Table 1).

TABLE 3

COMPOUN

Chemical na
Acetaldehyd
Acetic acid
Acetone
Acid red 27
Acridine
Actinomycin
Aldrin
Amitrole (3-
Aniline
Anthracene
Aspirin
Atrazine
Auramine
Bentazon
Benzylidene
2,2′-Biphen
Butyl alcoh
Butylated hy
Butylated hy
γ-Butyrolact
Cadmium ch
Cadmium su
Caffeine
Carbazol
Carbon tetra
Chloramphe
Chlorocholi
p-Chlorocin
(*Z*)-2-Chlor
Chloroform
3-Chloro-2-r
3-Chloronitr
3-Chloro-1-p
Chlorpyrifos
Chromium c
Chromium r
Chromium p
Cinnamalde
Cinnamic ac
Citrinin
Cyanazine
DDT
5,5′-Dibrom
Dicamba
2,4-Dichloro
5-(2,4-Dichl
Dieldrin
Diethyleneg
Diethyl stilb
p-Dimethyla
Dimethyl for
3,6-Dimethy
Dimethyl su
6,6′-Dinitrob
4,4′-Dinitrob

TABLE 3

COMPOUNDS GIVING A NEGATIVE RESPONSE IN BOTH THE SOS CHROMOTEST AND THE AMES TEST

Chemical name	SOS induction	Mutagenesis
Acetaldehyde	– (1)	– (b,1)
Acetic acid	– (1)	– (1)
Acetone	– (1)	– (1)
Acid red 27	– (1)	– (1)
Acridine	– (1)	– (1)
Actinomycin D	– (4)	– (b)
Aldrin	– (48)	– (a)
Amitrole (3-Aminotriazole)	– (2)	– (2,a,b)
Aniline	– (1)	– (a,b); + (1)
Anthracene	– (2,81)	– (a,2,81)
Aspirin	– (2)	– (2)
Atrazine	– (1,64)	– (1)
Auramine	– (2)	– (2,a); + (b)
Bentazon	– (64)	– (64)
Benzylidene acetophenone	– (74)	– (74)
2,2′-Biphenyl-dicarboxylic acid	– (58)	– (58)
Butyl alcohol	– (45)	– (a)
Butylated hydroxy-anisole	– (1,32)	– (a,1)
Butylated hydroxy-toluene	– (1,32)	– (a,1)
γ-Butyrolactone	– (2)	– (2)
Cadmium chloride	– (2)	– (2,b); T (a)
Cadmium sulfate	– (29,53)	– (b)
Caffeine	– (2)	– (2,a)
Carbazol	– (1)	– (1)
Carbon tetrachloride	– (51)	– (a)
Chloramphenicol	– (4)	– (a,b)
Chlorocholinchloride	– (1)	– (1)
p-Chlorocinnamic acid	– (74)	– (74)
(*Z*)-2-Chloro-3-(dichloromethyl)-butenedioic acid	– (52)	– (52)
Chloroform	– (2,51)	– (2,b)
3-Chloro-2-methyl-acrolein	– (78)	– (78)
3-Chloronitrobenzene	– (1)	– (1)
3-Chloro-1-propanol	– (1)	– (1); + (1)
Chlorpyrifos	– (64)	– (64); T (a)
Chromium chloride (CrIII)	– (16,29,53)	– (b); T (a)
Chromium nitrate (CrIII)	– (16,53)	– (b); T (a)
Chromium potassium sulfate (CrIII)	– (12,53)	– (b); T (a)
Cinnamaldehyde	– (74)	– (74)
Cinnamic acid	– (74)	– (74)
Citrinin	– (7,8)	– (8); T (a)
Cyanazine	– (64)	– (64)
DDT	– (1,2,23,28)	– (a,b,1,2,23)
5,5′-Dibromo-biphenyl-2,2′-dicarboxylic acid	– (58)	– (58)
Dicamba	– (48,64)	– (a,64)
2,4-Dichlorophenoxy-acid (2,4-D)	– (48)	– (a)
5-(2,4-Dichlorophenoxy)butyric acid (2,4-DB)	– (48)	– (a)
Dieldrin	– (2,28,48)	– (2,a,b)
Diethyleneglycol	– (1)	– (1)
Diethyl stilbestrol	– (23)	– (a,b,23)
p-Dimethylaminocinnamaldehyde	– (74)	– (74)
Dimethyl formamide	– (2)	– (2)
3,6-Dimethylphenanthrene	– (81)	– (81)
Dimethyl sulfoxide	– (2,4,8)	– (2,a)
6,6′-Dinitrobiphenyl-2,2′-dicarboxylic acid	– (58)	– (58)
4,4′-Dinitrobiphenyl-2,2′-dicarboxylic acid	– (58)	– (58)

TABLE 3 (continued)

Chemical name	SOS induction	Mutagenesis
Diphenylamine	– (1)	– (1); + (1)
Endrin	– (48)	– (a)
Epoxycyclododecane	– (59)	– (59)
1,2-Epoxy-5,9-cyclododecane-diene	– (59)	– (59)
Epoxycyclooctane	– (59)	– (59)
1,2-Epoxydecane	– (59)	– (59)
1,2-Epoxydodecane	– (59)	– (59)
1,2-Epoxyoctane	– (59)	– (59)
cis-2,3-Epoxysuccinic acid	– (59)	– (59)
Ethanol	– (1)	– (a,1)
Ethionine	– (2)	– (2,a)
Ethoxyquin	– (1,32)	– (1)
Ethyleneglycol	– (1)	– (1)
Fluorene	– (81)	– (a; 81)
9-Fluorenone	– (58)	– (58)
7-Cl-9-Fluorenone	– (58)	– (58)
9-Fluorenone-4-carboxylic acid	– (58)	– (58)
Glutathione	– (1)	– (1)
Griseofulvin	– (2,28,53)	– (2); T (a)
Hexachloroethane	– (51)	– (a)
Hexenal	– (78)	– (78)
Hydroxylamine	– (2)	– (2,a)
(+)-Limonene oxide	– (59)	– (59)
Methionine	– (2)	– (2)
Methoxychlor	– (1)	– (a,1)
o-Methoxycinnamaldehyde	– (74)	– (74)
Methylhydrazine	– (1)	– (1)
2-Methyl-1-nitronaphthalene	– (75)	– (75)
Metribuzine	– (64)	– (64)
Michler's ketone	– (1)	– (1)
Moniliformin	– (8)	– (8)
Naphthalene	– (81)	– (a; 81)
Naphthofuran: 5 derivatives	– (34)	– (34)
α-Naphthol	– (45)	– (a)
9-Nitroanthracene	– (75)	– (75)
4-Nitrobiphenyl-3′-carboxylic acid	– (58)	– (58)
5-Nitro-9-fluorenone-4-carboxylic acid	– (58)	– (58)
1-Nitronaphthalene	– (75)	– (75)
4-Nitrophenanthrenequinone	– (58)	– (58)
N-Nitroso-diphenylamine	– (1,2)	– (a,1,2); + (1)
Ochratoxin A	– (7,8)	– (8,b); T (a)
Patulin	– (7,8)	– (a,8)
Pentachloro-nitrobenzene	– (28)	– (28)
Pentenal	– (78)	– (78)
Perhexiline maleate	– (23)	– (23)
Phenanthrenequinone	– (58)	– (58)
Phenanthrenequinone-2-sulfonic cacid	– (58)	– (58)
α-Pinane oxide	– (59)	– (59)
Polychlorinated biphenyl (Aroclor 1254)	– (2)	– (2,b)
Prednisone	– (4)	– (b)
1-Propanol	– (1)	– (1)
Propionic acid	– (1,25)	– (1,25)
Propyl gallate	– (1,32)	– (1)
Quininehydrochloride	– (1)	– (1)
Quinoxaline	– (1)	– (1)

TABLE 3 (continued)

Chemical name	SOS induction	Mutagenesis
Rubratoxin B	– (8)	– (8)
Safrole	– (2)	– (2,a)
Sodium arsenate	– (29)	– (b)
Sodium arsenite	– (29)	– (b)
Sodium chloride	– (2,29)	– (2)
trans-Stilbene oxide	– (59)	– (59)
T2 toxin	– (7,8)	– (8)
1,1,2,2-Tetrachloroethylene	– (1)	– (b,1)
Thallium (1) acetate	– (1)	– (1)
Thallium (1) chloride	– (1)	– (1)
Tienillic acid (isomer 3)	– (23)	– (23)
Trenbolone	– (1,36)	– (1)
1,1,1-Trichloroethane	– (2,51)	– (2)
1,1,2-Trichloroethylene	– (1)	– (1)
Trifluralin	– (28)	– (a)
Tris-(hydroxymethyl)-methyl-2-aminomethane sulfone	– (2)	– (2)
Trypan blue	– (1)	– (a); + (1)
Urethane	– (2,23)	– (2,23)
Vincristine	– (4,43)	– (b)
Vitamin C	– (1,32)	– (a,1)
Zearalenone	– (7,8)	– (8); T (a)
Zeranol	– (1,36)	– (1)
Zirconium tetrachloride	– (60)	– (60)

For legend see Table 2.

nitro-*o*-phenylene-diamine, penicillinic acid and pyrene.

In fact, a clear-cut disagreement appeared for only 2 compounds. They are *N*-nitroso-diethylamine and *N*-nitroso-dimethylamine, which were reported negative by one laboratory (Von der Hude, 1988) whereas we found a strong positive response for the same 2 compounds (Quillardet et al., 1985). We have no explanation for this discrepancy.

In conclusion, the SOS chromotest is a highly reproducible test system. The number of conflicting results between different laboratories, which is already low, is even lower when the data are examined critically. We think that, if all compounds were tested using the same well defined procedure and, moreover, if strict common criteria to interpret the response were used, the test viability would largely exceed 90%.

Correlation between SOS induction measured in the SOS chromotest and mutagenesis measured in the Ames test

Mutagenesis data obtained in the Ames test were available for 482 compounds tested in the SOS chromotest. Data for the Ames test were taken, when available, from papers reporting SOS chromotest data on the same compounds, or from reference publications (McCann et al., 1975; Kier et al., 1986; IARC, 1987). In the latter two publications, we were surprised to see that essentially positive responses were taken into account by the authors. This means that several compounds found to be negative by numerous investigators were judged, in these publications, mutagenic in the Ames test, only because one positive response was reported by one investigator. Despite this debatable choice, we complied with the conclusions adopted in these papers (see discussion by Kier, 1988, p. 153).

From these 482 compounds, results from several laboratories were available for a number of compounds and contrasting responses were reported in one or the other test, for 73 compounds: 19 in the SOS chromotest; 62 in the Ames test; for 8 of these compounds conflicting results were reported in both tests. We decided to attribute a negative or a positive response to certain of these compounds according to the following rules: (i) the response given by the majority of the laboratories was selected; (ii) prefer-

ence was given to clear responses (+ or −) over doubtful or inconclusive responses (?, ± or T, see tables); (iii) preference was given to classical methods over modified methods, however if a positive result was obtained only in a modified method the response was taken into account. Thus comparisons of the results obtained in the Ames test to those obtained in the SOS chromotest could be made for 452 compounds. We could not conclude on a clear + or − response for 30 compounds.

Concordance between the two test systems

Out of 452 compounds tested, 373 (82%) gave similar responses in both tests: 236 positive responses (Table 2) and 137 negative responses concordant in both tests (Table 3). This result strengthens our previous conclusion, obtained from a smaller set of compounds, that there is a relatively close correlation between the induction of the SOS system as measured in the SOS chromotest and mutagenesis as measured in the Ames test (Quillardet et al., 1982, 1985). However in our previous study (Quillardet et al., 1985) a slightly higher correlation (89%) was obtained. This slight discrepancy could be due to the larger data base used here or to other causes such as compounds inadequately tested by some laboratories (in our previous study all of the compounds were tested in our laboratory), and, for the compounds which trigger only a slight increase of the SOS induction factor or a slight increase of the number of mutants, to a difference in the interpretation of test results.

Discrepancy between the two test systems

The discrepancies between both tests concern 79 compounds out of 452 (18%). Nineteen compounds were given as positive in the SOS chromotest and negative in the Ames test, and 60 compounds were given as negative in the SOS chromotest and positive in the Ames test.

Compounds giving a positive response in the SOS chromotest and a negative response in the Ames test

Nineteen compounds gave a positive response in the SOS chromotest and a negative response in the Ames test (Table 4).

TABLE 4

COMPOUNDS GIVING A POSITIVE RESPONSE IN THE SOS CHROMOTEST AND A NEGATIVE RESPONSE IN THE AMES TEST

Chemical name	SOS induction	Mutagenesis
Alachlor	+ (64)	− (64)
p-Aminocinnamic acid	+ (74)	− (74)
Amitraz	+ (39)	− (39)
Cacodylic acid	+ (64)	− (64); T (a)
(*E*)-2-Chloro-3-(dichloromethyl)-butenedioic acid	+ (52)	− (52)
Dihydroxyacetone	+ (1)	− (1)
3,3-Dimethylacrolein	+ (78)	− (78)
Dinoseb	+ (64)	− (64); T (a)
Estradiol-17β	+ (66)	− (b)
3-Ethoxy-2-methyl-acrolein	+ (78)	− (78)
3-Ethoxy-2-pentyl-acrolein	+ (78)	− (78)
3-Ethoxy-2-propyl-acrolein	+ (78)	− (78)
2-Furylacrolein	+ (74)	− (74)
Hydrogen peroxide	+ (1,61,63)	− (1)
Linuron	+ (64)	− (64); T (a)
5-Methoxybenzofuran (R7236)	+ (34)	− (34)
o-Nitrocinnamic acid	+ (74,78)	− (74,78)
Phenanthrene	+ (81)	− (a; 81)
Quinoline-1-oxide	+ (1)	− (1)

For legend see Table 2.

Since a fraction of mutagenesis is dependent upon the induction of the SOS system and in particular upon gene *umuDC*, responsible for SOS mutagenesis (see Walker, 1984), each chemical able to induce the SOS response would be expected to be mutagenic. Since inhibition of DNA synthesis is known to be sufficient to trigger the induction of the SOS system, compounds which are not direct genotoxic agents (i.e., they do not directly interact with DNA) but which can inhibit DNA synthesis through their action on enzymes of DNA metabolism are expected to induce the SOS response. However, in this case, the DNA being undamaged, mutagenesis would be untargeted, occuring only by induction of the SOS mutator effect (see Witkin, 1976; Walker, 1984). Untargeted mutagenesis is a minor component of mutagenesis which may yield effects at the limit of detection for the Ames test. This would explain why certain compounds (nondirect genotoxic agents which inhibit DNA synthesis) could induce the SOS system without being detected as mutagenic. One example of such a compound is nalidixic acid, an inhibitor of DNA gyrase. However, this compound appears in the present paper as an SOS inducer and mutagenic because it was reported to be slightly positive in the Ames test when assayed with the special strain TA102.

One can also imagine that certain DNA lesions which block DNA replication, and therefore are potentially able to induce the SOS response, cannot be converted into mutations. They could only be processed by an error-proof repair system or, if not, they would be lethal. Compounds inducing such lesions, if they exist, would be expected to be highly toxic.

On another hand, the differences in the methodology of both test systems could account for some positive response in the SOS chromotest of compounds which are not mutagenic in the Ames test. Indeed, results obtained in the SOS chromotest are relatively independent of the toxicity of the tested compound since survival of the bacterial tester strain is not required (see Quillardet et al., 1985). Thus, weak positive responses could be obtained in the SOS chromotest with toxic compounds which would not be detected in the standard Ames test, because they affect the growth of mutants. For example, a weak induction is observed in the SOS chromotest with dihydroxyacetone in the absence of metabolic activation (Von der Hude et al., 1988) and with phenanthrene in the presence of metabolic activation (Mersch-Sundermann et al., 1992). These two chemicals were not found to be mutagenic on standard Ames tester strains. However, using strain TA104, positive responses have been obtained with dihydroxyacetone (Marnett et al., 1985) and phenanthrene (Sakai et al., 1985) in the Ames test. Strain TA104 is more sensitive than other Ames tester strains to various aldehydes and ketones very toxic for the bacterial cell (Marnett et al., 1985). The use in the Ames test of special strains with a particularly sensitive target for mutagenicity, such as TA104, makes it possible to evidence the mutagenic effect of certain of these toxic compounds. Similarly, another compound toxic for the bacterial cell, hydrogen peroxide, presents an SOS inducing activity in the SOS chromotest, but is not mutagenic in the Ames test (Von der Hude et al., 1988; Eder et al., 1989c; Goerlich et al., 1989). Estradiol for which a strong toxic effect is observed after the peak of induction could belong to this category of compounds. Thus, the SOS chromotest could be particularly helpful to detect highly toxic genotoxic compounds of various chemical classes.

One or the other of these reasons could explain the relatively strong SOS induction observed in the SOS chromotest in the absence of activation mixture for quinoline-1-oxide for which the induction factor reaches more than 6 (Von der Hude et al., 1988), dinoseb for which the induction factor reaches more than 4, and linuron for which the induction factor is between 2 and 4 (Xu and Schurr, 1990). However, we may not exclude that other theoretical reasons, yet unsuspected, could account for these results and therefore, it will be interesting to study the mechanism of action of these compounds to really understand how they can induce the SOS system without leading to mutations.

At least, as already noticed (Quillardet et al., 1985), we cannot exclude that for compounds only active in the SOS chromotest in the presence of an activation mixture ((*E*)-2-chloro-3-(dichloromethyl)-butenoic acid and phenanthrene) the

TABLE 5

COMPOUNDS GIVING A NEGATIVE RESPONSE IN THE SOS CHROMOTEST AND A POSITIVE RESPONSE IN THE AMES TEST

Chemical name	SOS induction	Mutagenesis
Acephate	− (64)	+ (64)
Acridine flavine·HCl (proflavine)	− (1)	+ (1)
Aflatoxin G2	− (2,7)	+ (2); ? (b); T (a)
9-Aminoacridine	− (5,3,1) + / − (4)	+ (1,4)
o-Aminoazotoluene	− (4)	+ (a,4)
2-Aminopurine	− (2,24,45)	+ (2)
Benzidine	− (1,2,45)	+ (a,b,1,2)
1*H*-Benzotriazole	− (1)	+ (1)
Carbaryl	− (64)	− (64); + (a)
4′-Chloroacetyl-acetanilide	− (1)	+ (1)
2-Chloronitrobenzene	− (1)	+ (1)
4-Chloronitrobenzene	− (1)	+ (1)
Congo red	− (1)	+ (1)
Coronene	− (81)	+ (81)
Cyclohexenoxide	− (1)	+ (1)
Cyclophosphamide	− (1,2,4,43,53)	+ (a,b,1,2)
Diacetyl	− (1)	+ (1)
o-Dianisidine	− (1)	+ (a,1)
p-Dianisidine	− (1)	+ (1)
Dichloromethane	− (51)	+ (a)
1,2-Dichloropropane	− (1)	+ (a,1)
1,3-Dichloropropane	− (1)	+ (1)
2,3-Dichloropropanol	− (1)	+ (1)
1,3-Dichloropropanone	− (1)	+ (1)
Dimethoate	− (64)	+ (64)
4-Dimethylaminoazobenzene	− (1)	+ (a,1)
2,2-Dimethyl-1-methoxy-ethylene oxide	− (59)	+ (59)
4,4′-Dinitrobiphenyl-2,2′-dicarboxaldehyde	− (58)	+ (58)
Direct red 2	− (1)	+ (1)
cis-2,3-Epoxybutane	− (59)	+ (59)
Epoxycyclohexane	− (59)	+ (59)
Epoxycyclopentane	− (59)	+ (59)
1,2-Epoxyhexane	− (59)	+ (59)
exo-2,3-Epoxynorbonane	− (59)	+ (59)
1,2-Epoxy-7-octene	− (59)	+ (59)
1,2-Epoxypropane	− (59)	+ (59)
2,3-Epoxypropyl-(2-ethylhexyl)ether	− (59)	+ (59)
Ethidium bromide	− (3); + / − (4)	+ (4)
Ethylene dichloride	− (2,51)	+ (2,a)
Fast garnet GBC base	− (1)	+ (1); T (a)
Glutaraldehyde	− (1)	+ (1)
8-Hydroxyquinoline	− (45)	+ (a)
Kojic acid	− (8)	+ (8)
6-Mercaptopurine	− (4,45)	+ (a)
Methyl orange	− (1)	− (a); + (1)
Methyl red	− (1)	T (a); + (1)
Monocrotophos	− (64)	+ (64); T (a)
Mordant black 3	− (1)	+ (1)
1-(4-Nitrobenzol-azo)-2-naphthol	− (1)	+ (1)
Nitrofen	− (64)	+ (64)
Perylene	− (1,81)	+ (1,81)
PR toxin	− (8)	+ (8)
Sodium azide	− (1,2,4)	+ (a,1,2)

differences in the condition for metabolization inherent in the test systems are responsible for the different results observed (see also next section).

However, for most of the other compounds we think that more trivial reasons can explain why they were found not mutagenic in the Ames test but inducers of the SOS response in the SOS chromotest. Indeed these compounds promote only a very small increase of the induction factor. The interpretation will depend strongly on the criteria chosen to establish the judgment of positive or negative result. These compounds are alachlor and cacodylic acid (Xu and Schurr, 1990), 3,3-dimethylacrolein (Eder et al., 1990), estradiol (Van Aswegen et al., 1989), 3-ethoxy-2-methyl-acrolein and 3-ethoxy-2-pentyl-acrolein (Eder et al., 1990) for which the maximum induction factor is less than 2 in the absence of activation mixture. For several of these compounds a negative response was obtained in the presence of activation mixture. One can add to this series of compounds 3-ethoxy-2-propyl-acrolein for which the maximum inducing factor was 2.73 (Eder et al., 1990) and 5-methoxybenzofuran (R7236) for which the maximum induction factor was 2.12 in the absence of activation mixture and (*E*)-2-chloro-3-(dichloromethyl)-butenoic acid which is interpreted by the authors as only marginally active in the SOS chromotest (Tikkanen and Kronberg, 1990). In contrast, for similar reasons, these compounds, which may also have a low capacity to increase the mutation rate, could be under-estimated in the Ames test. Thus a reevaluation of the genotoxic activities of a number of these compounds could modify the interpretation of response for some of them and decrease the discrepancy between results in the two test systems.

The case of amitraz is peculiar. This compound was not found to be mutagenic in the standard Ames test on strains TA97, 98, 100, 102 with or without metabolic activation. It was not an SOS inducer in the SOS chromotest with or without metabolic activation on the classical strain PQ37. Thus there is good concordance between the results obtained in both tests. However, an SOS inducing activity was found in the SOS chromotest without metabolic activation on strain PQ243 which carries mutations *tagA* and *alkA* being thus deficient *N*-glycosylases (Tudek et al., 1988). It will be interesting to study the mutagenicity of amitraz in strains deficient in DNA glycosylases.

Insufficient data were available to us to discuss the case of *p*-aminocinnamic acid, *o*-nitrocinnamic acid and 2-furylacrolein (Eder et al., 1991b).

Compounds giving a negative response in the SOS chromotest and a positive response response in the Ames test

Sixty compounds gave a negative response in the SOS chromotest and a positive response in the Ames test (Table 5).

Since the induction of the SOS response is triggered by a replication block, compounds which induce lesions which do not perturb the DNA replication are not expected to induce the SOS response although they may be mutagenic. They create non SOS inducing miscoding lesions. This is likely the case for 2-aminopurine, a typical base analogue. Also, O6-alkylguanine is a lesion known

TABLE 5 (continued)

Chemical name	SOS induction	Mutagenesis
2,4,4′,6,-Tetranitrobiphenyl-2′,6-dicarboxylic acid (diamide)	− (58)	+ (58)
2,4,4′,6,-Tetranitrobiphenyl-2′,6-dicarboxylic acid (dimethyl ether)	− (58)	+ (58)
o-Toluidine	− (1)	T (a); + (b,1)
Trichlorfon	− (64)	+ (64,a)
1,2,3-Trichloropropane	− (1)	+ (1)
2,4,4′-Trinitrobiphenyl-2′-carboxylic acid	− (58)	+ (58)
2,4,4′-Trinitrobiphenyl-2′,6-dicarboxylic acid	− (58)	+ (58)

For legend see Table 2.

to be miscoding but which does not perturb DNA replication at least for short alkyl chains. This lesion is produced by alkylating agents such as ethyl or methyl methanesulfonate, N′-nitro-*N*-nitrosoguanidine. These compounds are SOS inducers because they create other lesions such as 3-methyladenine known to block DNA synthesis and to trigger the induction of the SOS system. However, one can imagine that some compounds produce only, or essentially, non SOS inducing miscoding lesions.

(1) Is there a correlation between base pair substitution mutation and SOS induction? It was noticed by several authors that most of the compounds which induce mutations in the Ames tester strain TA100, which is mainly reversed by base pair substitutions, are also SOS inducers in the SOS chromotest. In contrast, compounds which induce mutations only in Ames tester strains in which the *his*$^-$ mutation is a frameshift, TA98, TA1537 or TA1538, generally do not induce the SOS response in the SOS chromotest (see Von der Hude et al., 1988). One possible interpretation for this observation is that lesions which block the replicative complex induce the SOS system which makes it possible to bypass this replication block generating essentially base pair substitutions as mutations. On the contrary frameshift mutations could be due to lesions which do not block the replicative complex but which perturb it, promoting strand slipping inducing base additions or deletions. If this observation was valid, one would expect that most mutagenic compounds which do not induce the SOS response would be mutagenic in the frameshift strains TA98, TA1538, or TA1537, but not in the base pair substitution strains TA1535 or TA100.

This hypothesis is discussed below with 43 compounds for which sufficient data were available to us.

Sixteen of these compounds are better detected on frameshift strains TA98, TA1538, TA1537 than on base pair substitution strains. Thus, in the presence of metabolic activation mixture, benzidine is clearly mutagenic essentially on strain TA1538 and TA98 (Kier et al., 1986), *o*-dianisidine is mutagenic on strain TA98 and weakly, if at all, on strain TA100, but it is not mutagenic on strains TA1535, TA1537 (Haworth et al., 1983), and *p*-dianisidine is mutagenic on strains TA98, TA1538 and TA1537 (Dunkel et al., 1985). In the same conditions, kojic acid is mutagenic only on TA98 (Stark, 1980) and coronene on standard strain TA98 and on strain TA97 another frameshift strain, whereas it does not increase significantly the spontaneous mutation rate in TA100 (Sakai et al., 1985). Although the overall results for methyl red and fast garnet GBC base are 'T', i.e., inconclusive in the GeneTox publication (Kier et al., 1986), methyl red was found mutagenic in the presence of activation mixture on strain TA98 (Mori et al., 1986) and on strain TA1538 (Brown et al., 1978; Kier et al., 1986) and in the presence as well as in the absence of activation mixture. Fast garnet GBC base was found positive on strains TA1538 (Joachim et al., 1985) and TA98 (Longstaff et al., 1984). On other strains (TA100, TA1535 and TA1537) responses were inconclusive for these two compounds. In the absence of activation mixture, acridine flavine (proflavine) is best detected on strain TA1538 (McCann et al., 1975b), and 9-aminoacridine is efficiently detected on strain TA1538 but not on the other strains (Dunkel et al., 1984). Again, 4,4′-dinitrobiphenyl-2,2′-dicarboxaldehyde and 2,4,4′,6-tetranitrobiphenyl-2′,6-dicarboxylic acid (dimethyl ether) are mutagenic on strain TA1538 not on strain TA1535 (Vasilieva et al., 1990); 2,4,4′,6-tetranitrobiphenyl-2′,6-dicarboxylic acid (diamide), 2,4,4′-trinitrobiphenyl-2′-carboxylic acid and 2,4,4′-trinitrobiphenyl-2′,6-dicarboxylic acid are mutagenic on strains TA1538 and TA98 but not on strain TA1535 (Vasilieva et al., 1990). According to Von der Hude et al. (1990), 4′-chloroacetylacetanilide and 1-(4-nitrobenzol-azo)-2-naphthol are mutagenic on strains TA1538 and TA98. We do not know if these compounds require an activation mixture or not to be active nor if they are mutagenic on other strains.

By contrast, 21 compounds are better mutagens on the base pair substitution strain TA1535 or TA100 than on frameshift strains. Thus, in the presence of activation mixture positive responses were obtained for cyclophosphamide on strains TA1535 and TA100 whereas negative responses were obtained on TA98, TA1537, TA1538 (McCann et al.,
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Cann et al., 1975b; Kier et al., 1986). 8-Hydroxyquinoline is mutagenic on strain TA100 but not on strains TA1535, TA1537 or TA98 (Kier et al., 1986) and 1,3-dichloropropane is mutagenic on strain TA1535 whereas negative results were reported with strains TA98, TA1537, TA1538, and TA100 (Dean et al., 1985). 1,2,3-Trichloropropane is mutagenic on strains TA1535 and TA100 and weakly on strain TA98. It is not mutagenic on strain TA1537 (Haworth et al., 1983). Monocrotophos, trichlorfon and dimethoate are mutagenic on strain TA100 in the presence as well as in the absence of activation mixture but not on strain TA1535, TA1537, TA1538 or TA98 (Moriya et al., 1983; Haworth et al., 1983). Similarly, sodium azide and 1,2-epoxypropane were mutagenic on strains TA1535 and TA100 but not on strains TA98, TA1537, TA1538 (Gene-Tox, 1986; Dunkel et al., 1984; Bootman et al., 1979; Dean et al., 1985). Although responses obtained with 1,2-dichloropropane have been judged equivocal in a collaborative study (Haworth et al., 1983), this compound has been found mutagenic on strains TA1535 and TA100 both in the presence and in the absence of activation mixture but not on strains TA1537 or TA98 (De Lorenzo et al., 1977). In similar conditions 2,3-dichloropropanol is mutagenic on strains TA1535 and TA100 (Nakamura et al., 1979). In the absence of activation mixture, cyclohexenoxide and 6-mercaptopurine are mutagenic on strains TA1535 and TA100, but not on the frameshift strains TA1537 or TA98 (Jung et al., 1981; IARC, 1987). Again, 1,3-dichloropropanone is mutagenic on strain TA100 (Gold et al., 1978). 2,2-Dimethyl-1-methoxy-ethylene oxide, *cis*-2,3-epoxybutane, epoxycyclohexane, epoxycyclopentane, and exo-2,3-epoxynorbonane are mutagenic on TA100 and TA1535 (Frantz and Sinsheimer, 1981; Von der Hude et al., 1990). In addition, 1,2-epoxy-7-octene is mutagenic on TA100 but only weakly mutagenic on TA1535, and 1,2-epoxyhexane is weakly mutagenic on TA100 but not on TA1535 (Von der Hude et al., 1990).

Another set of 6 compounds was detected on base pair substitution strains TA100, TA1535 as well as on frameshift strain TA98, TA1538 or TA1537. Thus, in the presence of activation mixture ethidium bromide is clearly mutagenic on strains TA98, TA100, TA1538 and TA1537 (our unpublished results). In similar conditions, *o*-aminoazotoluene is mutagenic on frameshift strains TA1538 or TA98 and also on base substitution strain TA100 (McCann et al., 1975b), while dichloromethane is mutagenic on strains TA100 and TA1535 as well as on strain TA98. However, this compound has not been found mutagenic on strains TA1537 and TA1538 (Kier et al., 1986; IARC, 1987). Perylene has been found weakly mutagenic on strains TA1537, TA98 and TA100 and it is also clearly mutagenic on strain TA97 (Sakai et al., 1985). One can classify here other compounds such as nitrofen which has been found mutagenic on strain TA100, in the presence as well as in the absence of activation mixture and on strain TA98 only in the presence of metabolizing activation mixture although negative responses have been found on strains TA1535, TA1537 and TA1538 (Moriya et al., 1983), and 2,3-epoxypropyl-(2-ethylhexyl)-ether which is mutagenic on strains TA1538 and TA98 only in the presence of activation mixture and on strain TA100 in the presence as well as in the absence of activation mixture (Dean et al., 1985).

Therefore, in this series of 43 compounds, one can find compounds inducing mutations mainly on frameshift strains as well as compounds inducing mutations mainly on base pair substitution strains. Thus we do not confirm the hypothesis that compounds which induce mutations only in Ames tester strains in which the *his*$^-$ mutation is a frameshift, TA98, TA1537 or TA1538, generally do not induce the SOS response in the SOS chromotest.

(2) Weak and equivocal mutagens. Among the compounds giving a negative response in the SOS chromotest and a positive response in the Ames test, a large number are only extremely weak or equivocal mutagens.

(a) Weak mutagens. Several compounds may be considered extremely weak mutagens, if they are mutagenic at all. Thus aflatoxin G2 was found by us to induce a weak response on strain TA100 in the presence of activation mixture (Quillardet et al., 1982), whereas a response considered equivocal was given by McCann et al. (1975b). Two negative results obtained on strain TA98 in

the presence of activation mixture are reported in the Gene-Tox publication (Kier et al., 1986) but the compound was classified as 'T', i.e., results were judged insufficient to classify this compound as positive or negative in the Ames test. Acephate is judged to be mutagenic in the Ames test (Xu and Schurr, 1990). Indeed a positive response has been obtained on strain TA100 in the absence or in the presence of activation mixture. However, only a doubling of the spontaneous mutation rate is obtained at high concentrations of acephate (Moriya et al., 1983). Ethylene dichloride is an extremely weak mutagen which does not increase by more than 50% the spontaneous number of revertants on strain TA100 in the absence of activation mixture (McCann et al., 1975a). As mentioned by the authors, reproducible dose–response curves cannot be obtained. Other weak mutagens give a low response only under special conditions or on especially sensitive tester strains. Thus, methyl orange is not mutagenic on TA98, TA100, TA1535 and TA1537 when the standard procedure is used (Brown and Dietrich, 1983; Kier et al., 1986). Only a weak positive response was obtained when aerobic preincubation in the presence of riboflavin, which acts as an electron donor for the generation of azo radicals, was performed (Brown and Dietrich, 1983). This compound is judged nonmutagenic in the Gene-Tox publication (Kier et al., 1986). Similarly, for *o*-toluidine a positive response in the Ames test is given (Von der Hude et al., 1988) based on the results reported by Brown et al. (1983) who found a very weak mutagenic activity obtained with TA100 and TA98 under activation in anaerobic preincubation. However, *o*-toluidine is also reported negative in the standard Ames test (McCann et al., 1975b). In the Gene-Tox publication (Kier et al., 1986) negative responses are reported on strains TA100, TA1535 and TA1537 both with and without metabolic activation mixture and an inconclusive response on TA1538. However, the authors judged the data insufficient to classify the compound as negative or positive in the Ames test. Congo red and direct red 2 are weakly mutagenic on strains TA98 and TA100 only in the presence of activation mixture and if aerobic preincubation is performed (Brown and Dietrich, 1983). The mutagenicity of these compounds is increased when riboflavin, which acts as an electron donor for the generation of azo radicals, is added to the assay (Brown and Dietrich, 1983). A weak positive response has been obtained with mordant black 3 on strain TA100 under aerobic preincubation conditions. The compound is not mutagenic on strain TA98 in similar conditions (Brown and Dietrich 1983). In addition, PR toxin is a very weak mutagen which is detected only with the special strain TA 97 in which it increases the spontaneous mutation rate by only 60% (Levin et al., 1982). Diacetyl, which has not been found mutagenic on standard Ames tester strains, gave a weak positive response using strain TA104 which is more sensitive than other Ames tester strains to various aldehydes and ketones very toxic for the bacterial cell (Marnett et al., 1985).

(b) Equivocal mutagens. Other compounds may be considered equivocal mutagens. For these compounds contradictory results have been reported so that it is difficult to draw firm conclusions with regard to their mutagenicity in the Ames test. For example 2-chloronitrobenzene and 4-chloronitrobenzene are given as positive in the Ames test by the laboratory which evaluated their genotoxicity in the SOS chromotest. Data for mutagenicity came from a report by Shimizu et al. (1983) which found clear positive responses for 2-chloronitrobenzene in the absence of activation mixture on strains TA98 and TA1538, but negative responses on strains TA100 and TA1535, and clear positive responses for 4-chloronitrobenzene in the absence of activation mixture on strains TA100 and TA1535, but negative responses on strains TA98 and TA1538. They classify 2-chloronitrobenzene as a mutagen inducing frameshifts and 4-chloronitrobenzene as a mutagen inducing base-pair substitutions. However, these two compounds were evaluated in the Ames test by three different laboratories in a collaborative study (Haworth et al., 1983). In the absence of activation mixture 2-chloronitrobenzene was found negative on TA100 in 5 experiments, on TA1535 and TA1537 in 2 experiments and also in TA98. In the presence of activation mixture, using rat liver homogenate, 3 weak positive responses were found out of 5 experiments on strain TA100, 1 weak positive response out of 3 on strain TA98, and negative responses were reported on strains

TA1535 and TA1537. Using hamster liver homogenate, positive responses were found in all (5) experiments on strain TA100, 2 weak positive responses out of 3 on strain TA98, and negative responses were reported on strains TA1535 and TA1537. With 4-chloronitrobenzene, in the absence of activation mixture, negative responses were found on TA98, TA1537 and TA1535 and 1 weak positive response out of 4 was reported on TA100. This compound was found positive on TA100 and TA1535 with liver homogenate from rat and hamster, and a weak positive response out of 2 experiments was reported on strain TA98 with liver homogenate from hamster. Thus if testing conditions are varied a positive result in mutagenicity test for 2-chloronitrobenzene and 4-chloronitrobenzene can be found (Table 5).

In general, for these categories of weak or equivocal mutagenic compounds we think that the results have to be reevaluated. In particular, the tests have to be performed in parallel on the same batch of compound using similar conditions in both tests. We believe that a serious reevaluation of these compounds could lead to better agreement between both tests results. It is likely that some compounds which induce weak or equivocal genotoxic responses in the tests have been under- or over-estimated in one or the other test.

(c) Non standard strains or metabolization conditions. It should also be noted that several compounds which are considered here as positive in the Ames test would escape a classical screening using this assay. Indeed, these compounds are negative in standard test conditions with the standard tester strains, but give positive responses after adaptation of the assay or on especially sensitive tester strains. Thus, carbaryl was found negative in the Ames test by Moriya et al. (1983) but it was reported positive in the Gene-Tox publication although, if individual results are examined, it can be seen that carbaryl is given positive by only one laboratory. This result was obtained only on strain TA1538 and in the presence of uninduced mouse liver when a preincubation step is added to the assay. 4-Dimethylaminoazobenzene is given positive in the Ames test (Von der Hude, 1988). Indeed, a positive response has been obtained on TA98 and TA100 in the absence of activation mixture when an aerobic preincubation was performed (Brown and Dietrich, 1983). However, under standard conditions, 4-dimethylaminoazobenzene was not found mutagenic on strains TA98 and TA100 (Mori et al., 1986; Haworth et al., 1983) or on strains TA1535, TA1537, TA1538 (Haworth et al., 1983) in the absence or in the presence of metabolizing activation mixtures. According to the Gene-Tox publication (Kier et al., 1986) the overall results in the Ames test for 4-dimethylaminoazobenzol were positive in the presence of activation mixture. However, when individual results are detailed, it can be seen that only one positive response, obtained in the liquid preincubation test, is given out of 5 negative and one inconclusive responses from different laboratories. In addition, glutaraldehyde was not found mutagenic on standard Ames tester strains. However, using strain TA104 (Marnett et al., 1985) and TA102 (Levin et al., 1982), positive responses were obtained in the Ames test for this chemical.

As already mentioned, although almost identical activation mixtures are used, the conditions for metabolization may be quite different between the two tests. In the SOS chromotest metabolization occurs in liquid medium, whereas it occurs on plates in the standard Ames test. The agar medium used in the Ames test is believed to stabilize the activation enzymes and the time allowed for metabolic activation is longer in the Ames test than in the SOS chromotest. These differences in metabolic activation may account for several of the discrepancies observed between the two tests.

Correlation between SOS induction measured in the SOS chromotest, mutagenesis measured in the Ames test and carcinogenesis

A number of carcinogens are non-genotoxic carcinogens (see review by Butterworth, 1990) and therefore they will not be detected in genotoxicity tests such as the SOS chromotest or the Ames test. There are also practical reasons which can lead to an apparent absence of genotoxicity of a carcinogen which in fact would act by a genotoxic mechanism. One is that in a genotoxicity test a compound may not be transformed into

TABLE 6

COMPOUNDS OF CLASS 1

Chemical name	SOS induction	Mutagenesis
(a) Positive response in both the SOS chromotest and the Ames test		
2-Acetylaminofluorene	+ (1,3); +/− (4)	+ (a,1)
Adriamycin	+ (4,43)	+ (a,b)
Aflatoxin B1	+ (2,4,6,7,8,9,10,11...)	+ (2,a,b)
Aflatoxin B2	+ (2,7)	+ (2,b); T (a)
Aflatoxin G1	+ (2,4,7)	+ (2,b); T (a)
Aflatoxin M1	+ (2)	+ (a,2,b)
Benz[*a*]anthracene	+ (1,81)	+ (a,1,81)
Benzo[*b*]fluoranthene	+ (81)	+ (81)
Chromium oxide (CrVII)	+ (16)	+ (b)
Chrysene	+ (3,81)	+ (a,81)
Dibenz[*a*,*h*]anthracene	+ (81)	+ (a,81)
Dibenzo[*a*,*i*]pyrene	+ (81)	+ (a,81)
1,2-Dibromo-3-chloropropane	+ (3,28)	+ (a,b)
1,3-Dichloropropene	+ (1)	+ (a,b,1)
1,2:3,4-Diepoxybutane	+ (59)	+ (59)
1,2,3,4-Diepoxybutane	+ (1)	+ (a,1)
Diethyl sulfate	+ (1,2)	+ (1,2,a,b)
9,10-Dimethyl carbamoyl chloride	+ (2)	+ (2,a,b)
Dimethyl sulfate	+ (2)	+ (2,b); T (a)
Epichlorohydrin	+ (1,2,59,71,77)	+ (a,b,1,2,59)
Ethylenedibromide	+ (1,2,3,28)	+ (b,1,2); T (a)
Formaldehyde	+ (1)	+ (b,1); − (1)
Hydrazine	+ (2)	+ (2,b)
Metronidazole	+ (4,23)	+ (a,b,23)
1-Naphthylamine	+ (2)	+ (a,b)
2-Naphthylamine	+ (2,3)	+ (2,a,b)
Nitrogen mustard	+ (2)	+ (2,a,b)
N-Nitroso-dibutylamine	+ (2)	+ (2,a)
N-Nitroso-diethylamine	+ (2); − (1)	− (1); + (a,1,2)
N-Nitroso-dimethylamine	+ (2); − (1)	− (1); + (a,1,2)
N-Nitroso-dipropylamine	+ (2)	+ (a,2)
N-Nitroso-ethylurea	+ (1,2,3)	+ (a,1,2)
N-Nitroso-methylurea	+ (1,2,3,4,83)	+ (a,1,2)
N-Nitroso-morpholine	+ (2)	+ (2); T (a)
N-Nitroso-piperidine	+ (2)	+ (2,a)
1,3-Propane sultone	+ (2)	+ (2,a)
β-Propiolactone	+ (2,9)	+ (2,a)
Propyleneimine	+ (2)	+ (2,a)
Sodium dichromate (CrVI)	+ (12)	+ (a,b)
(b) Negative response in both the SOS chromotest and the Ames test		
Amitrole (3-Aminotriazole)	− (2)	− (2,a,b)
Auramine	− (2)	− (2,a); + (b)
Cadmium chloride	− (2)	− (2,b); T (a)
Cadmium sulfate	− (29,53)	− (b)
Carbon tetrachloride	− (51)	− (a)
Chloroform	− (2,51)	− (2,b)
DDT	− (1,2,23,28)	− (a,b,1,2,23)
Diethyl stilbestrol	− (23)	− (a,b,23)
Ethanol	− (1)	− (a,1)
Michler's ketone	− (1)	− (1)
Safrole	− (2)	− (2,a)
Sodium arsenate	− (29)	− (b)

TABLE 6 (continued)

Chemical name	SOS induction	Mutagenesis
Sodium arsenite	− (29)	− (b)
Urethane	− (2,23)	− (2,23)
(c) Different response in the SOS chromotest and the Ames test		
Alfatoxin G2	− (2,7)	+ (2); ? (b); T (a)
o-Aminoazotoluene	− (4)	+ (a,4)
Benzidine	− (1,2,45)	+ (a,b,1,2)
Cyclophosphamide	− (1,2,4,43,53)	+ (a,b,1,2)
o-Dianisidine	− (1)	+ (a,1)
4-Dimethylaminoazobenzene	− (1)	+ (a,1)
1,2-Epoxypropane	− (59)	+ (59)
Ethylene dichloride	− (2,51)	+ (2,a)
Fast garnet GBC base	− (1)	+ (1); T (a)
Nitrofen	− (64)	+ (64)
o-Toluidine	− (1)	T (a); + (b,1)
Estradiol-17β	+ (66)	− (b)

For SOS induction and mutagenesis see legend of Tables 1 and 2.
For carcinogenesis the classification was from Lewis (1991); Class 1: confirmed carcinogens (these substances are capable of causing cancer in exposed humans); Class 2: suspected carcinogens (these substances may be capable of causing cancer in exposed humans. The evidence is suggestive, but not sufficient to convince expert review committees); Class 3: questionable carcinogens (these entries have minimal published evidence of possible carcinogenic activity) (see text for additional information).

the active metabolite which is formed in vivo in the organism; another one is that certain compounds cannot penetrate into the tester cell.

It is interesting to assess the capacity of a given system to discriminate between known carcinogens and non-carcinogens. Terms have been proposed for describing the validity of short-term tests for carcinogen screening (Bedouelle and Hofnung, 1978; Cooper et al., 1979; Bartsch et al., 1980). The *sensitivity* reflects the capacity to identify carcinogens; the *specificity* the ability to discriminate between carcinogens and non-carcinogens; the *accuracy* the capacity to correctly identify both carcinogens and non-carcinogens.

The classification of Lewis (1991) was selected to classify chemicals as carcinogens. This classification of more than 3400 chemicals takes into account the OSHA (Occupational Safety and Health Administration), IARC (International Agency for Research on Cancer), ACGIH (American Conference of Governmental Industrial Hygienists) and DFG MAK (German Research Society's Maximum Allowable Concentration values) decisions. According to Lewis, substances are grouped into three classes: *class 1*, confirmed carcinogens (these substances are capable of causing cancer in exposed humans); *class 2*: suspected carcinogens (these substances may be capable of causing cancer in exposed humans; the evidence is suggestive, but not sufficient to convince expert review committees); and *class 3*: questionable carcinogens (these entries have minimal published evidence of possible carcinogenic activity) (for a detailed description see Lewis, 1991).

There is no class for non-carcinogens so that we have reevaluated the sensitivity of the SOS chromotest and the Ames test on the new data base, but not the specificity and the accuracy. In our previous study (Quillardet et al., 1985), on the basis of limited experimental results, we attempted to define non-carcinogens from the chemical series examined. Our classification allowed us to evaluate the specificity and the accuracy of both the Ames test and the SOS chromotest. Among the 73 chemicals tested, we found that the specificity was higher with the SOS chromotest (100%) than with the Ames test (62%). With the SOS chromotest a specificity of 100% was obtained because no false positives (e.g., non-carcinogen but SOS inducer) were found

among the 73 chemicals tested. It is unlikely that a specificity of 100% would be found on the larger number of compounds examined in this study. However, this result might indicate that induction of SOS is rather specific for carcinogens. The slightly higher accuracy obtained with

TABLE 7

COMPOUNDS OF CLASS 2

Chemical name	SOS induction	Mutagenesis
(a) Positive response in both the SOS chromotest and the Ames test		
Aflatoxicol	+ (7,8)	+ (8)
2-Aminoanthracene	+ (1,3,4,6,12,50,53...)	+ (a,1)
2-Aminofluorene	+ (1,2,3,12,67); − (4)	+ (a,1,4,67)
Azaserine	+ (2,3,4)	+ (2,a)
Benzo[*j*]fluoroanthene	+ (81)	+ (81)
Benzo[*a*]pyrene	+ (1,2,4,9,17,25,32,42...)	+ (a,1)
Bleomycin	+ (2,3,4,9,43,49)	+ (b)
Cisplatin	+ (4,18,19,31,43,79)	+ (b)
Dacarbazine	+ (43)	+ (b)
Daunomycin	+ (2,3,4,22,49,67,71)	+ (a,2,4,67)
Dibenzo[*a*,*h*]pyrene	+ (81)	+ (81)
7,12-Dimethylbenz[*a*]anthracene	+ (1,2,3,4,9,67,81)	+ (a,1,2,67,81)
1,3-Dinitropyrene	+ (75)	+ (75)
1,6-Dinitropyrene	+ (75)	+ (75)
1,8-Dinitropyrene	+ (75)	+ (75)
2,3-Epoxypropyl-phenylether	+ (59)	+ (59)
Ethyl methanesulfonate	+ (1,2,3,4,44,47)	+ (a,1,2)
Furylfuramide	+ (3,26,27)	+ (a)
Methylazoxymethanol-acetate	+ (2)	+ (2); T (a)
3-Methylcholanthrene	+ (1,2,3,9,67,71,81)	+ (a,1,2,67)
N-Methyl-*N'*-nitro-*N*-nitrosoguanidine	+ (1,2,3,4,20,21,39...)	+ (a,b,1,2)
Nitrofurazone	+ (14,62)	+(a,62)
2-Nitro-7-methoxynaphthofuran (R7000)	+ (2,34)	+ (2,34)
4-Nitro-*o*-phenylene-diamine	+ (67); − (3)	+ (a,67)
1-Nitropyrene	+ (3,65,75)	+ (65,75)
4-Nitroquinoline-*N*-oxide	+ (1,2,4,6,9,12,19,25...)	+ (a,1,2)
Sterigmatocystin	+ (7)	+ (a)
Styrene oxide	+ (59)	+ (59
2,4,7-Trinitro-9-fluorene	+ (67,71,75)	+ (67,75)
(b) Negative response in both the SOS chromotest and the Ames test		
Acetaldehyde	− (1)	− (b,1)
Aniline	− (1)	− (a,b); + (1)
Butylated hydroxy-anisole	− (1,32)	− (a,1)
Chloramphenicol	− (4)	− (a,b)
2,4-Dichlorophenoxy-acetic acid (2,4-D)	− (48)	− (a)
Ethionine	− (2)	− (2,a)
Griseofulvin	− (2,28,53)	− (2); T (a)
Methoxychlor	− (1)	− (a,1)
Methylhydrazine	− (1)	− (1)
Polychlorinated biphenyl (Aroclor 1254)	− (2)	− (2,b)
1,1,2,2-Tetrachloroethylene	− (1)	− (b,1)
1,1,2-Trichloroethylene	− (1)	− (1)
Trypan blue	− (1)	− (a); + (1)
(c) Different response in the SOS chromotest and the Ames test		
Dichloromethane	− (51)	+ (a)

For legend see Table 6.

the SOS chromotest (79%) as compared to the Ames test (75%) was essentially due to the larger number of false positives obtained with the Ames test.

Correlation between SOS induction, mutagenesis and confirmed carcinogens of class 1

Among 72 carcinogens of class 1, mutagenicity data were missing for 4 compounds evaluated only with the SOS chromotest, and for 3 compounds, we could not attribute a response + or − for mutagenicity. Thus the performance of the two tests could be compared on 65 compounds of class 1 (Table 6). 39 compounds (60%) gave a positive response in the two tests (Table 6a), 14 (22%) gave a negative response (Table 6b). For 12 compounds (18%) different responses were given by the two tests (Table 6c). Thus, the sensitivity, which reflects the capacity to identify carcinogens, was 62% with the SOS chromotest and 77% with the Ames test.

The SOS chromotest has a lower capacity to identify carcinogens of class 1 than the Ames test. In the previous section, we noticed that part of the disagreement between the two tests could be accounted for by compounds which give weak or equivocal responses in one or the other test. In agreement with this idea, 5 out of 12 compounds which yield a different response in the two tests are weak or equivocal genotoxic chemicals. Indeed, one compound, estradiol, is a very weak, and therefore an equivocal SOS inducer not detected as mutagen, and 4 compounds which are not detected in the SOS chromotest (aflatoxin G2, 4-dimethylaminoazobenzene, ethylene dichloride and *o*-toluidine) are very weak or equivocal mutagens which often are not detected in the standard Ames test.

The inability of other carcinogens of class 1, such as 9-aminoazotoluene, benzidine, cyclophosphamide, *o*-dianisidine, 1,2-epoxypropane, fast garnet GBC base or nitrofen, to induce the SOS response whereas they are undoubtedly mutagenic in the Ames test has been discussed in the previous sections. It may be due to the mechanism of genotoxicity of these compounds, or, for compounds which require metabolic activation to be active, to a different behavior of the activation mixture in the two assays.

Correlation between SOS induction, mutagenesis and suspected carcinogens of class 2

Among 62 carcinogens of class 2, mutagenicity data were missing for 15 compounds evaluated only with the SOS chromotest, and for 4 compounds, we cannot attribute a response + or − for mutagenicity. Thus the performance of the two tests could be compared on 43 compounds of class 2 (Table 7). 29 compounds of class 2 (67%) gave a positive response in the two tests (Table 7a), 13 (31%) gave a negative response (Table 7b) and for 1 compound (2%) a different response was given by the two tests. Thus, within this series of suspected carcinogens of class 2, there is an agreement of 98% between the results provided by the SOS chromotest and the Ames test, and the sensitivity, which reflects the capacity to identify carcinogens, was 66% with the SOS chromotest and 68% with the Ames test.

Therefore we can conclude that the SOS chromotest and the Ames test have a similar capacity to identify suspected carcinogens of class 2. Indeed, the discrepancy between the two tests was due to only one compound, dichloromethane, which is mutagenic in the Ames test but has not been found to induce the SOS response in the SOS chromotest.

Correlation between SOS induction, mutagenesis and both confirmed carcinogens of class 1 and suspected carcinogens of class 2

If one considers as carcinogens both confirmed carcinogens of class 1 and suspected carcinogens of class 2, one obtains a series of 108 compounds. 68 compounds of this series (63%) gave a positive response in the two tests, 27 (25%) gave a negative response and for 13 compounds (12%) different responses were given by the two tests. Thus, the sensitivity was 63% with the SOS chromotest and 73% with the Ames test.

Correlation between SOS induction, mutagenesis and questionable carcinogens of class 3

Among 135 questionable carcinogens of class 3, mutagenicity data were missing for 30 compounds evaluated only with the SOS chromotest, and for 10 compounds, we could not attribute a response + or − for mutagenicity or SOS induction. Thus the performance of the two tests could

TABLE 8

COMPOUNDS OF CLASS 3

Chemical name	SOS induction	Mutagenesis
(a) Positive response in both the SOS chromotest and the Ames test		
N-Acetoxy-2-acetylaminofluorene	+ (2)	+ (2,a)
Acridine (ICR170)	+ (1,3)	+ (a,1)
Acridine orange	+ (1,3,4,71)	+ (a,1)
Allyl-methanesulfonate	+ (44,47)	+ (44,47)
2-Amino-5-nitrothiazol	+ (1)	+ (1)
Anthanthrene	+ (81)	+ (a,81)
Benzo[*ghi*]perylene	+ (81)	T (a); + (81)
anti-Benzo[*c*]phenanthrene-3,4-diol-1,2-oxide	+ (68)	+ (68)
syn-Benzo[*c*]phenanthrene-3,4-diol-1,2-oxide	+ (68)	+ (68)
tert.-Butylhydroperoxide	+ (1,61)	+ (1)
Captafol	+ (3,28,64)	+ (64); T (a)
Captan	+ (3,28,64)	+ (64, a)
Cumene hydroperoxide	+ (1,61)	+ (1)
Dibenz[*a*,*c*]anthracene	+ (81)	+ (a,81)
1,2:5,6-Diepoxyhexane	+ (59)	+ (59)
1,2:7,8-Diepoxyoctane	+ (59)	+ (59)
9,10-Dimethyl-anthracene	+ (2)	+ (2)
N,*N*-Dimethyl-(2-chloro-ethyl)-amine	+ (1)	+ (1)
1,2-Epoxy-3-butene	+ (59)	+ (59)
N-Ethyl-*N'*-nitro-*N*-nitrosoguanidine	+ (1,3)	+ (1); T (a)
Ferbam	+ (64)	+ (64)
Fluoranthene	+ (81)	+ (81)
Folpet	+ (64)	+ (64,a)
Furazolidone	+ (3,62)	+ (62)
4-Hydroxyamino-quinoline-1-oxide	+ (3)	+ (a)
Hydroxyurea	+ (1,2,45,53)	+ (1,2)
9-Methylanthracene	+ (81)	+ (81)
Methyl methanesulfonate	+ (1,2,4,39,44,47...)	+ (a,1,2)
3-Nitrofluoranthene	+ (65,75)	+ (65,75)
2-Nitrofluorene	+ (2,3,4,9,65); − (1)	+ (1,2,4,75)
Nitrofurantoin	+ (14,15,26,27)	+ (a)
Potassium chromate (CrVI)	+ (6,16,29,53)	+ (a,b)
Potassium dichromate	+ (53)	+ (a,b)
Proflavin sulfate	+ (3)	+ (a)
Quercetin	+ (3,13,23,30)	+ (a,23)
Quinoxaline-1,4-dioxide	+ (1)	+ (1)
Triphenylene	+ (81)	+ (81)
(b) Negative response in both the SOS chromotest and the Ames test		
Acid red 27	− (1)	− (1)
Actinomycin D	− (4)	− (b)
Aldrin	− (48)	− (a)
Anthracene	− (2,81)	−(a,2,81)
Atrazine	− (1,64)	− (1)
Butylated hydroxy-toluene	− (1,32)	− (a,1)
γ-Butyrolactone	− (2)	− (2)
Caffeine	− (2)	− (2,a)
Carbazol	− (1)	− (1)
Chlorocholinchloride	− (1)	− (1)
3-Chloronitrobenzene	− (1)	− (1)
Chromium chloride (CrIII)	− (16,29,53)	− (b); T (a)
Chromium nitrate (CrIII)	− (16,53)	− (b); T (a)
Chromium potassium sulfate (CrIII)	− (12,53)	− (b); T (a)
Citrinin	− (7,8)	− (8); T (a)

TABLE 8 (continued)

Chemical name	SOS induction	Mutagenesis
Dieldrin	– (2,28,48)	– (2,a,b)
Diethyleneglycol	– (1)	– (1)
Dimethyl sulfoxide	– (2,4,8)	– (2,a)
Endrin	– (48)	– (a)
1,2-Epoxydodecane	– (59)	– (59)
1,2-Epoxyoctane	– (59)	– (59)
Ethyleneglycol	– (1)	– (1)
9-Fluorenone	– (58)	– (58)
Hexachloroethane	– (51)	– (a)
Naphthalene	– (81)	– (a,81)
N-Nitroso-diphenylamine	– (1,2)	– (a,1,2); + (1)
Ochratoxin A	– (7,8)	– (8,b); T (a)
Patulin	– (7,8)	– (a,8)
Pentachloro-nitrobenzene	– (28)	– (28)
Phenanthrenequinone	– (58)	– (58)
Prednisone	– (4)	– (b)
1-Propanol	– (1)	– (1)
Propyl gallate	– (1,32)	– (1)
1,1,1-Trichloroethane	– (2,51)	– (2)
Trifluralin	– (28)	– (a)
Vincristine	– (4,43)	– (b)
Zearalenone	– (7,8)	– (8); T (a)
(c) Different response in the SOS chromotest and the Ames test		
Acridine flavine·HCl (proflavine)	– (1)	+ (1)
1*H*-Benzotriazole	– (1)	+ (1)
Carbaryl	– (64)	– (64); + (a)
2-Chloronitrobenzene	– (1)	+ (1)
4-Chloronitrobenzene	– (1)	+ (1)
Coronene	– (81)	+ (81)
Cyclohexenoxide	– (1)	+ (1)
1,2-Dichloropropane	– (1)	+ (a,1)
Dimethoate	– (64)	+ (64)
Epoxycyclohexane	– (59)	+ (59)
8-Hydroxyquinoline	– (45)	+ (a)
6-Mercaptopurine	– (4,45)	+ (a)
Methyl red	– (1)	T (a); + (1)
Perylene	– (1,81)	+ (1,81)
PR toxin	– (8)	+ (8)
Sodium azide	– (1,2,4)	+ (a,1,2)
Trichlorfon	– (64)	+ (64,a)
Cacodylic acid	+ (64)	– (64); T (a)
Dinoseb	+ (64)	– (64); T (a)
Hydrogen peroxide	+ (1,61,63)	– (1)
Phenanthrene	+ (81)	– (a,81)

For legend see Table 6.

be compared on 95 compounds of class 3. 37 compounds of class 3 (38.5%) gave a positive response in the two tests (Table 8a), 37 (38.5%) gave a negative response (Table 8b) and for 21 compound (22%) different responses were given by the two tests (Table 8c). In this series the sensitivity of both assays decreased. It was 43% with the SOS chromotest and 57% with the Ames test. This is not surprising since compounds of class 3 are questionable carcinogens for which

TABLE 9

COMPOUNDS TESTED IN THE SOS CHROMOTEST FOR WHICH MUTAGENESIS OR CARCINOGENESIS DATA WERE NOT AVAILABLE

Chemical name	SOS induction
2-Acetyl-1-dodecyl-5-nitronaphthofuran	− (34)
Acridine yellow	+ (1,45)
Ajmalicine	− (56)
Allopurinol	− (45)
Alodan	− (48)
Aluminum chloride	− (29)
Aluminum sulfate	− (29)
1-Amino-4-nitronapthalene	+ (75)
Ammonium sulfide	− (29)
Ampicillin	− (53); +/− (4)
Ascorbate-copper (oxidized solution)	+ (38)
Atrapazine	− (48)
Averufin	− (7)
8-Azaguanine	− (45)
3′-Azido-3′-deoxythymidine (AZT)	+ (55)
Azobisisobutyronitrile	+ (61)
Barium nitrate	− (29)
Bismuth nitrate	− (29)
5-Bromo-2′-deoxyuridine	+ (45) (24); − (1)
Calcium nitrate	− (29)
Carminomycin	+ (22)
CB 1954 (nitroimidazole-aziridine drugs)	+ (46)
Cesium sulfate	− (29)
CHIP (platinum complex)	+ (79)
Chlorbenside	− (48)
3-Chloroacrolein	+ (78)
Chlorodibromomethane	− (51)
2-Chloro-3-methylacrolein	+ (78)
4-(4-Chloro-2-methylphenoxy)butyric acid (MCPB)	− (48)
4-Chlorophenoxyacetic acid (4-CPA)	− (48)
6-Chlorothymine	− (45)
5-Chlorouracil	− (45)
Chlorthiamide	− (48)
Chromomycin	− (4)
Citreoviridin	− (8)
Colistin	+/− (4)
Cyclocytidine HCl	+ (45)
Daunomycin: 8 analogues	+ (45)
Daunomycinone	− (22)
Daunomycinone: 4 derivatives nos. 10, 11, 13, 15	+ (22)
Daunomycinone: 1 derivative no. 14	− (22)
DDT-DDVP mixture	+ (45)
Diacetoxyscirpenol	− (7)
3,6-Diamino-10-methylacridine	− (1)
Diazouracil	+ (45)
cis-1,2-Dichloroethylene	− (51)
trans-1,2-Dichloroethylene	− (51)
2,4-Dichlorophenoxy acetic acid methyl ester (2,4-DE)	− (48)
2′,3′-Dideoxyadenosine (ddA)	+ (55)
2′,3′-Dideoxycytidine (ddC)	− (55)
2′,3′-Dideoxy-2′,3′-didehydrothymidine (D4T)	+ (55)
2′,3′-Dideoxyguanosine (ddG)	+ (55)
2′,3′-Dideoxyinosine (ddI)	+ (55)
2′,3′-Dideoxythymidine (ddT)	+ (55)

TABLE 9 (continued)

Chemical name	SOS induction
2,7-bis-(2-*N*,*N*-Diethylamino-ethoxyl)-1,8-diazafluo	+ (17)
2,7-Dinitronaphthalene	+ (75)
2,4-Dinitrophenyl thiocyanate	+ (3,28)
Diplodiol	− (8)
Diplosporin	− (8)
Dodecyl gallate	− (1,32)
Ducitol	+ (45)
Ethylene-diamine malonatoplatinum II (JM 40)	+ (31)
Ethyl vinyl ketone	+ (76)
Fast blue RR	+ (45)
Fecapentaene-12	+ (73)
Fecapentaene-14	+ (73)
Ferric chloride	− (29)
Ferric nitrate	− (29)
Ferric sulfate	− (29)
Formic acid	− (1)
Fulvic acid	+ (82)
Furylethylene: 28 derivatives	+ (26,27)
Furylethylenes: 6 derivatives	− (26,27)
Gentamicin	− (4)
Gentisic acid	+ (82)
Gliotoxin	− (8)
Hexoestrol dibromoacetate	− (45)
Humic acid	+ (82)
2-Hydroxyestradiol	+ (66)
Iodine	− (29)
Iproplatin	+ (31)
Magnesium chloride	− (29)
Magnesium sulfate	− (29)
Mercuric chloride	− (29)
Methanol	− (1)
2-Methylphenanthrene	+ (81)
Mithramycin	− (4)
Molybdenum pentachloride	− (29)
Naphthofuran: 3 derivatives	+ (34)
β-Naphthol	− (45)
Nigeron	− (8)
Nitracine	+ (4)
4-(4-Nitrobenzyl)-pyridine	− (1)
Nitrocaphane	+ (45)
2-Nitro-9-fluorenone	+ (75)
5-Nitro-2-furfurylnitrate	+ (83)
5-Nitro-2-furylacrylic acid	+ (21,83)
2-(5-Nitro-2-furyl)-5-methoxy-benzofuran	+ (34)
2-Nitro-7-methoxynaphthofuran (R7000): coumarinic analogue	+ (34)
2-Nitronaphthofuran: 21 derivatives	+ (34)
5-Nitro-1-naphthonitrile	+ (3)
Nogalamycin	− (22)
Norsolorinic acid	+ (7)
Novobiocin	− (4)
Octylgallate	− (1,32)
9-Oxo-10-acridine acetic acid	− (17)
9-Oxo-10-acridine butyric acid	− (17)
Oxoplatin	+ (79)
Paraplatin	+ (31)

TABLE 9 (continued)

Chemical name	SOS induction
Paraquat (methylviologen)	– (61)
Phleomycin E	+ (5)
trans-Platinous diamine chloride	– (79)
Potassium chloride	– (29)
Potassium ferricyanide	– (29)
Potassium ferrocyanide	– (29)
Potassium iodide	– (29)
Potassium perchlorate	– (29)
Potassium permanganate	– (29)
Psoralen (in the dark)	+ (33)
Pyrethrin	– (45)
Pyridoxine hydrochloride	– (9)
Razoxanum	+ (45)
Roseotoxin	– (8)
RSU1069 (radiosensitizer)	+ (70)
RSU1069 (nitroimidazole-aziridine drugs)	+ (46)
RSU1069 (6 analogues)	+ (70)
Sodium bicarbonate	– (29)
Sodium carbonate	– (29)
Sodium hydrogenophosphate	– (29)
di-Sodium hydrogenophosphate	– (29)
Sodium molybdate	– (29)
Sodium nitrilotriacetate	– (53)
Stannous chloride	+ (29)
Streptonigrin	+ (4,49,55)
Tetracycline	– (4)
Tilorone	+ (17)
TMA (platinum complex)	+ (79)
α-Tocopherol	– (32)
Trichlorofluoromethane	– (51)
Trioxalen (in the dark)	+ (33)
Vamidothion	+ (28)
Vanillin	+ (45)
Veractamycin A (PD 114, 759)	+ (49)
Veractamycin B (PD 115, 028)	+ (49)
Versicolorin A	+ (7)
Versiconol acetate	– (8)
Viomellein	+ (8)
Vitamin E	– (1)
Vitamin K1/ferric chloride	+ (80)
Vivakorfen	+ (17)
Xanthomegnin	– (8)
Zinc acetate	– (29)

For legends see Tables 1 and 2.

data are not sufficient to consider them definitely carcinogenic. It is likely that, in this series, after reevaluation, several compounds will be recognized as non-carcinogens.

Conclusions

From all these data, it appears that there is a relatively close correlation between the induction

of the SOS system as measured in the SOS chromotest and mutagenesis as measured in the Ames test. Out of 420 compounds, 341 (81%) gave similar responses in both tests. The discrepancy between results of both tests may often be accounted for by a difference in the interpretation of the results rather than by a difference in the experimental results themselves so that the correlation between both tests might be higher. This confirms that with very few exceptions, most mutagenic genotoxins are also SOS inducers (Quillardet et al., 1982, 1985).

The capacity to identify carcinogens, the sensitivity, of the Ames test (class 1: 77%; class 2: 68%) is higher than the sensitivity of the SOS chromotest (class 1: 62%; class 2: 66%). Thus the Ames' test may appear, in that respect, a better assay than the SOS chromotest for the screening of potential carcinogens. However, we noticed (Quillardet et al., 1985) that the specificity, i.e., the ability to discriminate between carcinogens and non-carcinogens, could be higher with the SOS chromotest than with the Ames test: a specificity of 100% was obtained with the SOS chromotest among 73 chemicals examined. This means that the Ames test is likely to yield more false positives than the SOS chromotest (Quillardet et al., 1985). Because in Lewis' classification (Lewis, 1991) non-carcinogens are not considered, the specificity of the tests was not reevaluated in the present study. However, the better sensitivity of one test and the better specificity of the other one suggest that they may be used in a complementary way for carcinogen screening. In addition, the minor differences observed between the results of both assays may also complement the deficiencies of one or the other test. For these reasons we think that the SOS chromotest may be useful as a complement to the Ames test in a battery of short-term tests for carcinogens.

The SOS chromotest is probably the most simple and rapid short-term test. These advantages are of great interest since they allow a good reproducibility of the assay, and easy adaptations to the monitoring of genotoxic agents in complex mixtures or in particularly unfavorable media. They have also allowed the automation of the SOS test which is particularly suitable for a large-scale screening of genotoxic agents. In addition, it may be recalled that the results given by the test are quantitative, allowing the classification of the genotoxic agents according to their SOSIP, and that some additional mechanistic information on the tested chemicals can be obtained by using some strains derived from the standard tester strain (Quillardet and Hofnung, 1988). In further reviews, it would in particular be interesting to compare quantitative genotoxicity data with quantitative carcinogenicity data (Peto et al., 1984; Gold et al., 1984).

In summary, as already proposed, the SOS chromotest could be a useful complement to the Ames test in at least three ways. First, because of its simplicity and rapidity, it could be used as an early assay in the screening of potential carcinogens. Second, it might allow the detection of genotoxic chemicals inactive in the Ames test (e.g., estradiol). Third, it may make it possible to discriminate false positives in the Ames test.

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