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GENOTOXICITY AND TOXICITY OF FOUR CHROMIUM COMPOUNDS MEASURED IN BACTERIAL SYSTEMS

by

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ABSTRACT

Cr(VI) compounds ($K_2Cr_2O_7$ and CrO_3) were confirmed to cause significant gene mutations in Salmonella mutagenicity tests but were only weak inducers of the SOS repair systems in the SOS Chromotest. Cr(III) compounds ($CrK(SO_4)_2$ and $CrCl_3$) while negative in the Salmonella system produce strong induction in the SOS Chromotest. The addition of S-9 mix decreases the four chromiums' genotoxicity in both testing systems and almost eliminates Cr(III) compounds' inducibility in the SOS Chromotest.

In the SOS Chromotest, chromium activity after 60 minutes of colour development, became relatively stable and it was found that a chemical's inducibility is independent of the absolute activity of the two enzymes - β-galactosidase and alkaline phosphatase - as long as the enzymes' ratios remain the same.

The four chromium compounds were found to have low inducing potency (SOSIP) as they only exhibit inducibility at very high concentrations. These four compounds produced toxic effects at lower concentrations in the ATP-TOX System than in the Microtox test. Cr_2O_3 is the most toxic chromium compound in all test systems and $CrK(SO_4)_2.12H_2O$ is the least toxic. The addition of S-9 mix also decreases the inhibition of protein biosynthesis by the chromium compounds.

In $CrK(SO_4)_2.12H_2O$ and $CrCl_3.6H_2O$ the chromium atoms appear to act in the same manner in exerting their genotoxic and toxic effects on the organisms and the other compound constituents appear to be inactive. The possible genetic damaging and toxic mechanisms of the four chromium compounds are discussed.

SOMMAIRE

D'après les épreuves effectuées sur la bactérie Salmonella, les composés de Cr(VI) ($K_2Cr_2O_7$ et CrO_3) peuvent provoquer d'importantes mutations génétiques. Par ailleurs, l'épreuve SOS Chromotest a révélé que ces composés n'exercent qu'un faible pouvoir inducteur sur les systèmes de réparation SOS. Par contre, les composés de Cr(III) ($CrK(SO_4)_2$ et $CrCl_3$) ont donné des résultats négatifs dans l'épreuve de dépistage des effets mutagènes et se sont avérés de puissants agents inducteurs dans l'épreuve SOS Chromotest. Si on ajoute du mélange S-9 aux échantillons, la génotoxicité des quatre composés s'en trouve réduite et le pouvoir inducteur des composés de CR(III) mesuré au moyen du SOS Chromotest devient presque nul.

Dans le SOS Chromotest, l'activité du chrome s'est stabilisée à la fin d'une période de développement des couleurs de soixante minutes. On a découvert que le pouvoir inducteur des composés ne dépend pas du tout du taux d'activité absolue des deux enzymes B-galactosidase et alcalino phosphatase, tant que celles-ci sont présentes en proportion constante.

Les quatre composés semblent posséder un pouvoir inducteur (SOSIP) assez faible puisque celui-ci ne se manifeste qu'à des concentrations extrêmement élevées. L'épreuve ATP-TOX a permis de mesurer les effets toxiques des quatre composés à des concentrations inférieures à celles que nécessite l'épreuve Microtox. D'après l'ensemble des épreuves, le Cr_2O_3 apparaît comme le composé le plus toxique et le $(CrK(SO_4)_2.12H_2O$ le moins toxique. L'ajout de mélange S-9 ralentit l'effet inhibiteur des composés de chrome sur la biosynthèse des protéines.

Dans le $(CrK(SO_4)_2.12H_2O$ et le $CrCl_3.6H_2O$, les atomes de chrome semblent exercer leurs effets toxiques et mutagéniques sur les organismes de la même manière tandis que les autres éléments constitutifs des composés semblent inactifs. L'étude comporte une discussion des mécanismes possibles par lesquels les composés de chrome peuvent causer des effets toxiques et des dommages génétiques.

MANAGEMENT PERSPECTIVE

Great Lakes water quality reports indicate that sediment loadings of chromium exceed MOE guidelines for Lake Superior, Lake Ontario and Lake Huron. These findings confirm that chromium compounds are widespread, widely used and will continue to be a potential health problem of unknown magnitude.

This study examined the toxicity and genotoxicity of four chromium compounds using the SOS Chromotest and ATP-TOX biomonitoring screening procedures. It was found that the compounds exhibit general toxicity at concentrations lower than those at which genetic damage occurs. Thus indicating that both toxicity and genotoxicity must be considered in the environmental assessment of chromium compounds.

This work is part of the NWRI Biomonitoring Project to develop and extensively evaluate a battery of microbiological and biochemical screening assays for routine applicatio in water quality monitoring. The tests are rapid, inexpensive and provide an indication of the overal toxicity of an environmental sample or mixture of chemicals rather than the concentration of individual contaminants. A carefully selected battery of such tests will compliment and improve the efficiency of traditional water quality monitoring activities.

PERSPECTIVE GESTION

D'après les rapports sur la qualité des eaux des Grands lacs, il semble que les charges de chrome dans les sédiments des lacs Supérieur, Ontario et Huron dépassent les lignes directrices du ministère de l'Environnement. Cette constatation confirme que les composés de chrome sont répandus dans le milieu, qu'ils sont très employés et qu'ils risquent de poser pour la santé un problème durable dont on ignore les proportions.

Les auteurs de la présente étude ont examiné la toxicité et la génotoxicité de quatre composés de chrome au moyen des épreuves de dépistage SOS Chromotest et ATP-TOX. Ils ont constaté que la toxicité globale des composés analysés se manifestent à des concentrations inférieures à celles qui engendrent normalement des dommages génétiques. Ces résultats indiquent qu'on doit donc tenir compte à la fois de la toxicité et de la génotoxicité de ces composés pour évaluer leur incidence sur le milieu.

Cette étude se rattache aux travaux du Projet de biosurveillance de l'Institut national de recherche sur les eaux. Celui-ci a pour but d'élaborer et d'évaluer de façon exhaustive une batterie d'épreuves microbiologiques et biochimiques qui pourraient servir à la surveillance régilière de la qualité de l'eau. Ces épreuves rapides et économiques permettent de connaître la toxicité globale d'un échantillon renfermant plusieurs composés chimiques différents sans avoir à déterminer la concentration respective de chacun. En constituant une batterie d'épreuves triées sur le volet, l'INRE sera à même de perfectionner les techniques classiques de surveillance de la qualité de l'eau et de les appliquer avec plus d'efficience.

INTRODUCTION

Chromium is one of the many chemicals, found in the Canadian Great Lakes System with known effects in mammals, that are currently subjected to regulatory monitoring in Canada and the U.S.A. (Committee on the assessment of human health effects of Great Lakes water quality, 1981). In earlier studies, 1977, sediment loadings of chromium far exceeded Ministry of Environment (Ontario) guidelines of 25 ppm, e.g. Lake Superior 163 ppm, Lake Ontario 48 ppm and Lake Huron 32 ppm. (Report on Great Lakes Water Quality, Appendix, 1981). These findings confirm the belief that chromium compounds are widespread, widely used and will continue to be a potential health problem of unknown magnitude.

Epidemiological studies have long shown that workers involved in the chromium industry have an increased risk of developing lung cancer (Machle and Gregorium, 1948; Brinton et al., 1952; Davies, 1978). Some hexavalent chromium compounds have been shown to induce tumors in some strains of mice (Roe and Carter, 1969; Nettesheim and Hammons, 1971). Hexavalent chromium compounds have also been shown to be mutagenic in bacterial and yeast systems (Nishioka, 1975; Lofroth and Ames, 1977; Bonatti et al., 1976). Other genotoxicity tests such as infidelity of DNA replications in vitro (Bianchi et al., 1983), cell transformation of hamster fibroblasts (Levis et al., 1978) and sister-chromatid exchanges in different rodent cell cultures (Bianchi et al., 1983) have been positive for hexavalent chromium.

Data have also shown that trivalent chromium [Cr(III)] was generally negative in genotoxicity assays unless a direct interaction with purified DNA was permitted by the test conditions (Flessel, 1979; Leonard and Lauwerys, 1980; Bianchi et al., 1983).

Recently Quillardet et al. (1982) developed an assay (the SOS Chromotest) which directly measures the damage to DNA through the actions of the SOS DNA repair system. In this test, genotoxicity is evaluated by the SOS response of E. coli to DNA damaging agents which results in a rapid biosynthesis of the enzyme - β-galactoridase whose concentration can be determined colorimetrically after the addition of ONPG (o-nitrophenyl-β-D-galactoside). Even at toxic doses, genotoxicity can still be measured by correcting (or normalizing) the β-galactosidase activity by alkaline phosphatase activity (representing protein biosynthesis activity) which is determined by the addition of PNPP (p-nitrophenyl phosphate).

In this report we present the results obtained from the application of a modified SOS Chromatest, performed in 96-well microplates, on four chromium compounds. The SOS Chromotest genotoxicity data are compared with those obtained from the Salmonella mutagenicity test (Maron and Ames, 1984).

Although many studies have been carried out to evaluate the genotoxicity of chromium compounds, rarely have their toxicity to bacterial systems been assessed. Here, also reported are the results of the toxic activity of four chromium compounds in three bacterial systems, Microtox test, SOS Chromotest and the newly developed ATP-TOX System (Xu and Dutka, 1987c).

MATERIALS AND METHODS

Chemicals:

The following chromium compounds were tested: CrO_3 (B.D.H.); $CrCl_3.6H_2O$, $CrK(SO_4)_2.12h_2O$ and $K_2Cr_2O_7$ (Baker Chemical Co., Phillipsburg, N.J., U.S.A.).

Test Systems:

A. Salmonella Mutagenicity Test:

The test was performed following the preincubation procedure detailed by Maron and Ames (1984). Salmonella typhimurium TA-98 and TA-100 were used. The S-9 fraction was purchased from Litton Bionetics, Inc., Charleston, S.C., U.S.A.

B. SOS Chromotest:

Reagents:

Bacterium E. coli K-12 PQ37 from Organics Ltd. Israel; L.B. Medium: Bacto typtone 10 g, Bacto yeast extract 5 g, NaCl 10 g/L distilled water; B buffer: Na_2HPO_4 16.1 g, $NaH_2PO_4.H_2O$ 5.5 g, KCl

0.75 g, $MgSO_4.7H_2O$ 0.25 g, sodium dodecyl sulfate 1 g, β-mercapto-ethanol 2.7 mL/L distilled water, adjusted to pH 7.0; P buffer: tris (hydroxymethyl) aminomethane ($C_4H_{11}NO_3$) 121 g, sodium dodecyl sulfate 1 g/L distilled water and adjusted pH 8.8 with HCl; ONPG (o-nitrophenyl-β-D-galactoside solution (4 mg/mL) and PNPP (p-nitrophenyl phosphate) solution (4 mg/mL) (both from Sigma) were prepared freshly by dissolving 80 mg PNPP into 20 mL B and P buffer, respectively.

Procedures:

(1) Thaw a vial of frozen E. coli K-12 PQ37 and inoculate into a 125 mL Erlenmyer flask containing 50 mL L.B. medium and 20 μg/mL ampicillin. Incubate at 37°C for 17 hours on a rotary shaker (100 rpm).

(2) After incubation, transfer 1 mL of the culture into 50 mL fresh L.B. medium and continue to incubate at 37°C for 30 min.

(3) Dispense 10 μL of serially-diluted chromium compound solutions into the wells (three wells for each dilution) of a microplate (96 wells, sterile, flat bottom). Two microplates were prepared in exactly the same manner, one for β-galactosidase activity measurements (expression of induction, IND), the other for alkaline phosphatase activity measurements (expression of protein biosynthesis, to check toxicity, TOX). Sterile distilled water was used as the spontaneous induction control (four wells) and one corner well was left empty to obtain a machine blank reading.

(4) Dispense 100 µL of the 30 min culture into each well of both IND and TOX plates. Seal the plates in a plastic bag to avoid unbalanced air flow and put the plate into a 37°C incubator with rotary shaking (100 rpm) for 2 hours.

(5) After 2 hours exposure of the bacteria to the chemicals, check the background reading of all wells at 410 nm on Minireader II (Dynatech Laboratories, Inc.) and then add ONPG solution and PNPP solution (100 µL/well) to the IND plate and TOX plate, respectively and incubate all plates at room temperature for colour development.

(6) After 15, 30, 60, 90, 120 min of colour development, check the optical density of every plate at 410 nm on the Minireader.

(7) For SOS Crhomotest with S-9 activation, S-9 mix is added to the bottle of bacterial culture (1 volume of S-9 mix plus 3 volumes of bacterial culture) just prior to dispensing into the wells of both IND and TOX plates. The other procedures are the same as the above. 2-AA (2-amino anthracene) and 4NQO (4-nitroquinoline 1-oxide) were used as postive controls for tests with and without S-9 addition, respectively.

Results Expression:

The enzyme units, induction factor and SOSIP were calculated according to the formula described by Quillardet et al. (1985a). The parameters of a specific period of time for each compound were calculated from corrected readings, e.g. subtract 15 minute reading

from the 30 minute reading (net colour increase) to calculate the β-galactosidase unit value during the period of 15 to 30 min (average); subtract 60 min reading from 90 min reading to produce the β-galactosidase units during the 60 to 90 min incubation period for colour development.

Protein biosynthesis inhibition (alkaline phosphatase inhibition) was calculated as follows:

$$I(\%) = \frac{C-U}{C} \times 100\%$$

C: alkaline phosphatase activity for spontaneous induction (distilled water control);

U: alkaline phosphatase activity for a chemical (or a sample) at a specific concentration.

C. Microtox Test:

The Microtox test was performed using the luminescent bacterium Photobacterium phosphoreum and following the procedure detailed in Beckman Microtox System Operation Manual (1982) with contact time of up to 15 minutes. The Microtox EC_{50} is defined as the concentration of toxicant causing a 50% reduction in light output from the base level.

D. ATP-TOX System:

The concentration of ATP per bacterial cell remains relatively stable throughout all phases of growth (D'Eustachio and Johnson, 1968). Thus, bacterial densities can be easily estimated by measuring the ATP content of the test system. When rapidly growing bacterial cells are exposed to toxicants, growth inhibition usually occurs. After several life cycles, the toxic effect can be estimated by comparing sample cell growth to the control via ATP content. However, some toxicants not only inhibit bacterial growth, but also affect the luciferase acitivity during ATP determinations. Therefore, the observed light output reduction of the test system is the net result of the inhibition of both bacterial growth and luciferase (called "total inhibition" of the ATP-TOX System). Luciferase activity inhibition can be determined by adding a standard ATP solution, as enzyme substrate, to the sample and to a distilled water control and measuring the light emission of the enzyme.

The ATP-TOX System used in this study was based on the use of E. coli K-12 PQ37 and following the procedure detailed by Xu and Dutka (1987c).

RESULTS AND DISCUSSION

Previous studies have shown that Cr(VI) compounds (such as $K_2Cr_2O_7$ and CrO_3) were able to induce gene mutations in Salmonella typhimurium, whereas Cr(III) compounds [such as $CrK(SO_4)_2$ and $CrCl_3$] usually do not induce mutations (Petrilli and de Flora, 1977; Bianchi et al., 1983). This study on the mutagenicity of the four different chromium compounds, as measured by the Salmonella mutagenicity test with preincubation, confirms the above earlier findings. From Fig. 1, it can be seen that two Cr(III) compounds [$CrK(SO_4)_2.12H_2O$ and $CrCl_3.6H_2O$] do not induce significant mutations in both TA-98 and TA-100 either with or without S-9 addition. However, the Cr(VI) compounds ($K_2Cr_2O_7$ and CrO_3) tested induced significant mutations in both strains. These Cr(VI) compounds at concentrations ranging from 50 to 200 µg/plate produced very high numbers of revertant colonies, much higher than previously reported (Petrilli and de Flora, 1977; Bianchi et al., 1983), probably due to the preincubation procedure used in this study which increased the response of the bacteria to the chemicals. It can be also seen that the addition of S-9 usually produces an apparent decrease in the number of revertant colonies by Cr(VI) compounds, thus supporting the results observed by Bianchi et al. (1983) and other researchers (Gruber and Jennette, 1978; Petrilli and de Flora, 1978). Salmonella typhimurium TA-98 shows more tolerance to Cr(VI) compounds than TA-100; whereas TA-100 seems to be less sensitive to Cr(III) compounds.

Contrary to Salmonella mutagenicity test results, Cr(III) compounds show a strong inducibility in the SOS Chromotest, while Cr(VI) compounds, which are strongly positive in the Salmonella system, are very weak inducers in the SOS Chromotest (Fig. 2). It can also be seen in Fig. 2 that the addition of S-9 mix decreases inducibility of all four chromium compounds tested. In Fig. 2, for each chromium compound, β-galactosidase activity (representing expression of sfiA gene) and alkaline phosphatase activity (representing cell protein biosynthesis rate) are shown on the left (a,c,e,g), and the resultant induction factors are shown on the right (b,d,f,h), both with and without S-9 addition. As Cr(VI) concentrations increase, bacterial β-galactosidase activity also slightly increases and the alkaline phosphatase activity decreases slowly (see Fig. 2 a,c). The resultant induction factors, which are the corrected ratios of the the enzymes' activities, slightly increase and finally decrease when the chemical concentrations are high enough to produce a toxic effect (Fig. 2, b,d). In testing $CrK(SO_4)_2.12H_2O$ and $CrCl_3.6H_2O$ [Cr(III)],without the addition of S-9, the β-galactosidase activities initially increase as Cr(III) concentrations increase. Approaching a specific concentration, the alkaline phosphatase activities start to drop (indicating a toxic effect), but β-galactosidase activities still keep increasing (Fig. 2, e,g) and do not start to decrease until more than 75% of the protein biosynthesis was inhibited (see Fig. 6). The resultant induction

factor curves become peak-shaped (Fig. 2, f,h). Interestingly, if the chemical concentrations are changed from total W/V to Cr atomic W/V, the average peak areas lie within a very similar concentration range for $CrK(SO_4)_2$ and $CrCl_3$. This suggests that Cr(III) atoms in these two compounds act similarly in inducing genetic damage and exerting a toxic effect. The remainder of the compound contributes little to the chromium compounds inducibility and toxicity. However, when S-9 mix is added, β-galactosidase activities only slightly increase, and both β-galactosidase and alkaline phosphatase activities start to decrease at the same chemical concentration for either $CrK(SO_4)_2.12H_2O$ or $CrCl_3.6H_2O$. It was found that the addition of S-9 not only decreases Cr(III)'s inducibility [β-galactosidase activities were much lower with S-9 than without the S-9 (compared to the control level)] but also ameliorates chromium's toxicity (alkaline phosphatase activity started to decrease at higher concentration with S-9 than without the S-9). Consequently, the induction factor of Cr(III) with the addition of S-9 remained very low (Fig. 2, f, h).

Most genotoxicity studies conducted on chromium compounds demonstrated that Cr(VI) compounds are generally positive and Cr(III) compounds are negative (Nishioka, 1975; Lofroth and Ames, 1977; Flessel, 1979; Leonard and Lauwerys, 1980; Bianchi et al., 1983). Our study on chromium compounds using the SOS Chromotest points to a completely reverse phenomenon: Cr(III) compounds are strongly positive inducers whereas Cr(VI) are weak but significant inducers.

These different results can be explained by the fact that the SOS Chromotest, which is based on the bacterial repair system, is a totally different system from most of the well-established genotoxicity screening tests (such as Salmonella mutagenicity test, gene mutations in cultured hamster cells, sister chromatid exchanges in cell cultures). The SOS repair system mainly detects DNA structure aberations (such as DNA strand breaks, crosslinks), while the typical mutation test, e.g. Salmonella mutagenicity test, mainly screens for point or base-shift mutations on normal DNA molecules. Based on our results and data, we suggest that Cr(VI) and Cr(III) compounds act differently in the reaction with DNA. When Cr(VI) compounds briefly make contact with the DNA, this mainly causes point or baseshift mutations (DNA structure remains normal after these mutations), and occasionally brings about some crosslinks between DNA strands. Thus, Cr(VI) compounds show strong positive activity in Salmonella or other mutagenicity tests but only weak activity in the SOS Chromotest (Fig. 2, b,d). On the other hand, Cr(III) compounds react with DNA and frequently cause strand breakage and crosslinks and/or they may become DNA-Cr(III) complexes. Thus the SOS repair system is able to detect these DNA structure alterations and strongly responds by activating the expression of the β-galactosidase gene. Whereas in Salmonella typhimurium strains, for example, even though Cr(III) compounds may produce point or other kinds of mutation, it is still impossible for the bacterial cell to express its histidine independence because

Cr(III) compounds may still keep combining tightly to the DNA or the DNA remains broken. Thus, the bacterial cell remains auxotrophic in phenotype (Fig. 1). Bianchi et al. (1983) found that Cr(III) compounds, when tested on purified DNA, produced DNA strand breaks as well as DNA crosslinks, whereas Cr(VI) brought about crosslinks only. This supports our findings and indicates that the strong induction response brought about by Cr(III) compounds in the SOS Chromotest is mainly due to DNA strand breaks and the DNA crosslink is a minor contributor to SOS repair system induction.

Bianchi et al. also noticed that Cr(III) was negative in seven mutagen screening test systems except when directly placed in contact with purified DNA molecules. They suggested that the lack of effect on intact mammalian cells can be attributed to the inability of Cr(III) to pass through the cell membranes. Interestingly, our study on intact bacterial cells showed that Cr(III) compounds could pass through bacterial cell membranes. This difference in membrane permeability may be the reason that Cr(III) was negative in mammalian cell DNA repair system (Bianchi et al., 1983).

Fig. 3 shows Cr(III) inducibility at different colour development time intervals. In Fig. 3 can be seen that maximum induction occurred after 30 minutes of colour development for most of the concentrations tested. At very low concentrations, e.g. 91 mg/L for both chemicals, induction factors are less than 1 (theoretically, induction factors should be ≥ 1), resembling the phenomenon observed earlier with weakly

inducible environmental samples (Xu and Dutka, 1987a). These surprising and unusual results imply that the data obtained after only 30 minutes colour development, are not stable. However, induction factors from 60, 90, 120 minutes colour development appear to be stable for all chromium concentrations tested. As a consequence, 90 minutes was selected as typical colour development time.

Response variations of three independent SOS Chromotest trials on $CrK(SO_4)_2.12H_2O$ are shown in Fig. 4. The β-galactosidase and alkaline phosphatase activity for Experiment 1 and 2 are similar, whereas the enzyme activities obtained from Experiment 3 (which was conducted using ONPG and PNPP solutions commercially available from Organics Ltd., Israel) were much higher than those in Experiment 1 and 2 (Fig. 4A). This implies that ONPG and PNPP solutions from Organics Ltd. are more efficient than laboratory prepared solutions in determining β-galactosidase and alkaline phosphatase activity. Nevertheless, the resulting induction factor dose-response curves obtained from these three independent tests are identical (Fig. 4B). This finding indicates that inducibility is independent of the absolute activity of the two enzymes. At specific chemical concentrations, the two enzymes' corrected ratios remain the same. Thus, the SOS Chromotest procedure has very good reproducibility.

Compared to the SOS Chromotest, which provides two key quantitative parameters, induced gene expression and protein biosynthesis, most other genotoxicity evaluation systems are

relatively less quantitative. For example, if a chemical is able to show its genetic damaging ability only after the dose used is higher than the minimum toxic concentration, the Salmonella mutagenicity test would not detect this chemical as a positive as most of the bacteria would be unable to form visible colonies or even be killed before a reaction could be manifested. However, in the SOS Chromotest, even at toxic concentrations, genotoxicants can be detected by determining the ratios between the two enzymes' activities and obtaining an induction factor higher than 1.5 (Fig. 2, e,f,g,h).

Table 1 presents the SOS Inducing Potency (SOSIP) of the four chromium compounds. It can be seen that the SOSIP values are low for all four compounds, even for the two strongly genotoxic compounds: $CrK(SO_4)_2.12H_2O$ and $CrCl_3.6H_2O$. For $K_2Cr_2O_7$ and CrO_3, low SOSIP values are easily understood; for $CrK(SO_4)_2$ and $CrCl_3$, low SOSIP were obtained because they were positive for genotoxicity only at very high concentrations: higher than 300 and 200 mg/L for $CrK(SO_4)_2.12H_2O$ and $CrCl_3.6H_2O$, respectively (Fig. 2, f,h). The SOSIP of 4NQO and 2AA are 33.00 and 2.15, respectively, which are much higher than those shown by the chromium compounds. These compounds also start to show inducibility at very low doses (0.05 mg/L for 4NQO and 1.25 mg/L for 2AA) (Xu and Dutka, 1987). Although Cr(III) compounds are strongly genotoxic at high concentrations, when compared with 4NQO and 2AA, they must be classifed as weak inducers.

The toxic activity of chromium compounds as shown by the Microtox and ATP-TOX Systems is demonstrated in Fig. 5. These two test systems are believed to be the most sensitive bacterial toxicity screening tests (Xu and Dutka, 1987b). It can be seen in Fig. 5 that the four chromium compounds exhibit typical dose-response toxic activity and these compounds produce toxic effects at lower concentrations in the ATP-TOX System. A very unusual phenomenon was observed while determining the four Cr compounds' toxicity via the Microtox test at different exposure times (Table 2). It can be seen that the four Cr compounds showed typical time-course effects (Xu and Dutka, 1987b) in the ATO-TOX System. In the Microtox test, the toxic effect of the four Cr compounds on light emission by Photobacterium phosphoreum decreases as exposure time increases. This phenomenon may be attributed to the testing of the four compounds via the Microtox procedure without pH adjustment (Table 3). Possibly the Photobacteria were inhibited due to their sudden exposure to the acidic chromium compounds. Later the bacterial buffer system came into effect, reducing the acidic effects portion of the Cr compounds' toxicity and increasing bacterial activity and thus the bacterial light emission. ATP-TOX System is a relatively long-term test (5 h). Bacterial buffering action might have occurred shortly after the bacteria were exposed to the acidic Cr compounds, but growth inhibition after a few cell cycles dominated the whole system, giving rise to a typical time-course effect in the ATP-TOX System. We did not adjust the pH of

the chromium solutions to 6.8 when performing the Microtox test as recommended, because the test results then would not be comparable with those obtained via the ATP-TOX System.

Inhibition of E. coli protein biosynthesis by the four chromium compounds is summarized in Fig. 6. Protein biosynthesis is shown by the alkaline phosphatase activity portion of the SOS Chromotest. Figure 6 data indicate that S-9 addition decreases chromium's toxicity to protein biosynthesis and at very low concentrations, some chromium compounds slightly stimulate protein biosynthesis, i.e. $K_2Cr_2O_7$, $CrK(SO_4)_2$ and $CrCl_3$ when added with S-9 and $CrK(SO_4)_2$ without S-9.

The EC_{50} or IC_{50} of the four chromium compounds in different toxicity screening systems are clarified in Table 4. Here it can be seen that CrO_3 is the most toxic chromium in all the toxicity screening test systems and $CrK(SO_4)_2.H_2O$ is the least toxic. If we look at chromium's net concentration (figures in brackets), $CrK(SO_4)_2.12H_2O$ and $CrCl_3.6H_2O$ have very similar toxicities, indicating that Cr atoms (or ions) in these two compounds act in the same manner in demonstrating their toxicity, which is similar to their exhibition of inducibility. Among the toxicity screening procedures evaluated, the ATP-TOX System (total inhibition, 5 h) appears to be the most sensitive system to determine chromium's toxicity. The luciferase assay in ATP-TOX System showed that the Cr compounds do not noticeably inhibit luciferase activity at low concentrations. Thus the total inhibition of the ATP-TOX System by Cr compounds may be mainly due to growth inhibition. Although the toxicity evaluation

procedure in the SOS Chromotest is not very sensitive, it is a relatively simple and rapid (2 h) test to perform, and the resulting data are critical in calculating a chemical or a sample's inducibility.

CONCLUSIONS

Two Cr(VI) compounds ($K_2Cr_2O_7$ and CrO_3) gave strong positive responses in the Salmonella mutagenicity test, while the two Cr(III) compounds [$CrK(SO_4)_2.12H_2O$ and $CrCl_3.6H_2O$] were positive in the SOS Chromotest. These findings suggest that both Cr(VI) and Cr(III) compounds have genotoxicity and they act differently in exerting their effects. Further research is required to reveal the induction mechanism of Cr(III) in the SOS Chromotest as well as in other systems.

The four chromium compounds exhibit their toxicity at concentrations lower than those at which genetic damages occur. Thus, precautions should be taken not only against the genotoxicity of chromium compounds but also against their general toxicity - physical or physiological.

The SOS Chromotest is an excellent system to detect genotoxicity and toxicity. It is fast (6 hours), efficient, accurate and fully quantitative. This test is not only comparable to other established genotoxicy systems (Quillardet et al., 1985), but also provides an

important addition to other systems in genotoxicity evaluation. Based on the data obtained during this study, to avoid false and inappropriate judgements, it is recommended that a battery of tests be used to assess the toxicity or genotoxicity of chemicals or environmental samples.

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TABLE 1. SOS-inducing potency of four chromium compounds.

Compound	SOSIP*	
	Without S-9	With S-9
$K_2Cr_2O_7$	0.022	0.010
CrO_3	0.058	0.034
$CrK(SO_4)_2.12H_2O$	0.093	0.002
$CrCl_3.6H_2O$	0.063	0.001

*Unit is 'per nmol'.

TABLE 2. Toxicity of four chromium compounds in Microtox and ATP-TOX system at different exposure times.*

Compounds	Microtox T_2C_{50}			ATP-TOX IC_{50}		
	5 min	10 min	15 min	1.2 h	3.5 h	5 h
$K_2Cr_2O_7$	65.0	68.0	68.0	138.0	35.0	24.0
CrO_3	10.4	12.1	12.5	50.0	3.9	3.8
$CrK(SO_4)_2.12H_2O$	120.0	128.0	130.0	55.0	37.0	47.5
$CrCl_3.6H_2O$	57.5	62.5	65.0	30.0	17.5	27.0

*EC_{50} or IC_{50} unit is mg/L.

TABLE 3. pHs* of four chromium solutions at different concentrations (mg/L).

$K_2Cr_2O_7$		CrO_3		$CrK(SO_4)_2.12H_2O$		$CrCl_3.6H_2O$	
Concen.	pH	Concen.	pH	Concen.	pH	Concen.	pH
12.50	5.37	3.13	4.77	37.50	4.48	37.50	4.42
25.00	5.16	6.25	4.35	75.00	4.25	75.00	4.25
50.00	4.96	12.50	3.91	150.00	4.06	150.00	4.08
100.00	4.76	25.00	3.61	300.00	3.87	300.00	3.91
200.00	4.65	50.00	3.33	600.00	3.70	600.00	3.72

*Chemicals were diluted using Microtox diluent and every solution contains a specific concentration of NaCl in order to facilitate metabolism of Photobacterium phosphoreum.

TABLE 4. Sensitivity of three toxicity screening procedures to four chromium compounds.

Compound	Microtox EC_{50}	ATP-TOX System IC_{50} (E. coli)		SOS-TOX IC_{50} (E. coli)(30 min)	
	(15 min)	Total Inhibition (5 h)	Luciferase Inhibition	-S-9	+S-9
$K_2Cr_2O_7$	68.0* (23.8)**	24.0 (8.4)	2680.0 (938.0)	154.6 (54.1)	163.7 (57.3)
CrO_3	12.5 (6.5)	3.9 (2.0)	1250.0 (650.0)	62.1 (32.3)	109.1 (52.7)
$CrK(SO_4)_2.12H_2O$	130.0 (13.5)	47.5 (4.9)	4320.0 (449.3)	327.3) (34.0)	654.6 (68.1)
$CrCl_3.6H_2O$	65.0 (13.0)	27.0 (5.4)	1980.0 (396.0)	190.8 (38.2)	408.8 (81.8)

*Total compounds concentration (mg/L).
**Figures in brackets are net chromium concentration (Cr mg/L).

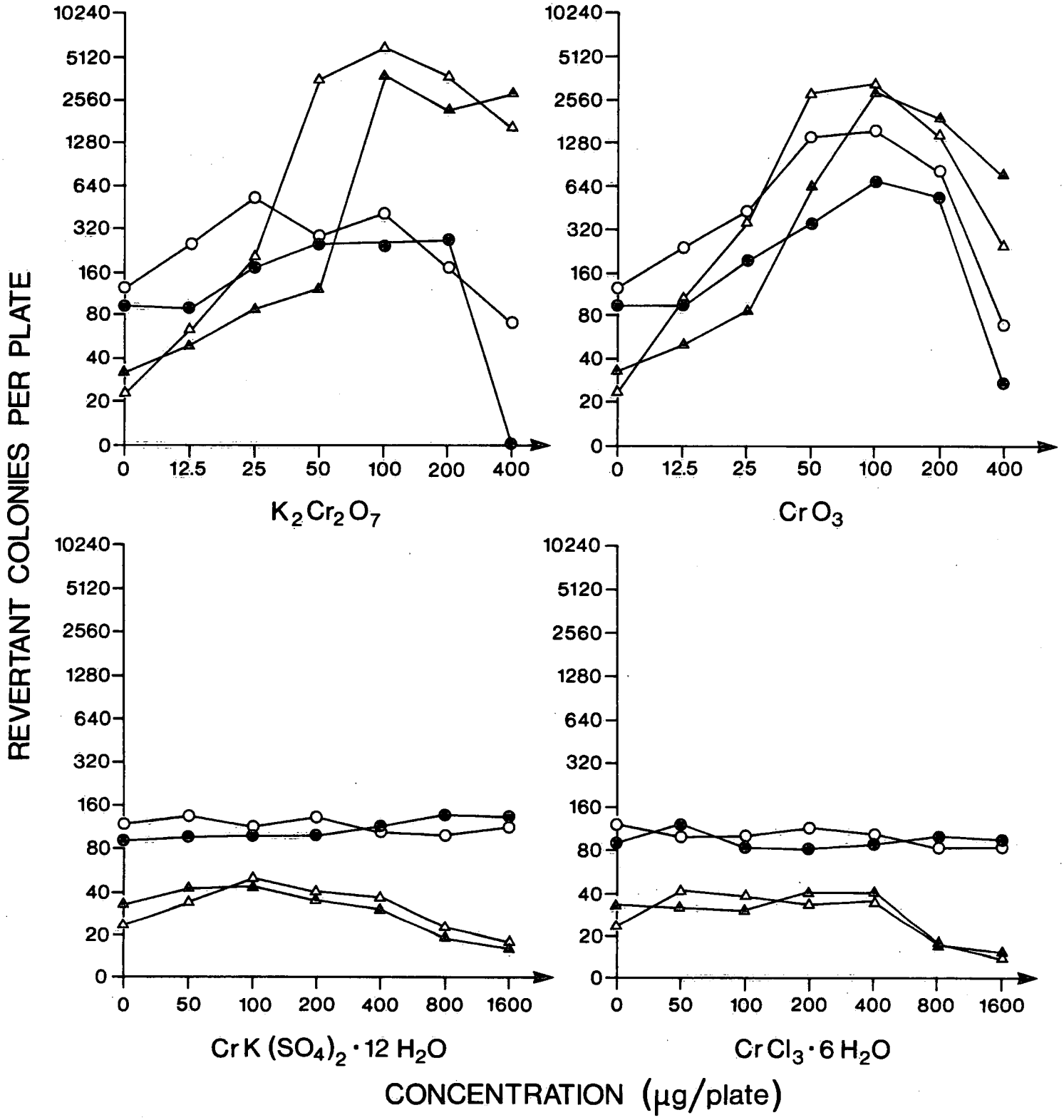

FIGURE 1. AMES' TEST DOSE-RESPONSE CURVES OF FOUR CHROMIUM COMPOUNDS

△——△ TA-98 ○——○ TA-100

▲——▲ TA-98+S-9 ●——● TA-100+S-9

Figure 3. Inducibility and toxicity of four chromium compounds [$K_2Cr_2O_7$ (A)(B); CrO_3 (C)(D); $C_2K(SO_4)_2.12H_2O$, (E)(F); $CrCl_3.6H_2O$, (G)(H)] in SOS Chromotest. β-galactosidase activity (square) and alkaline phosphatase activity (triangle) are shown in (A)(C)(E)(G). Induction factors (circle) are shown in (B)(D)(F)(H). Open symbols represent Chromotest without S-9, closed symbols represent Chromotest with S-9. The figure was drawn from 90 min colour development time data.

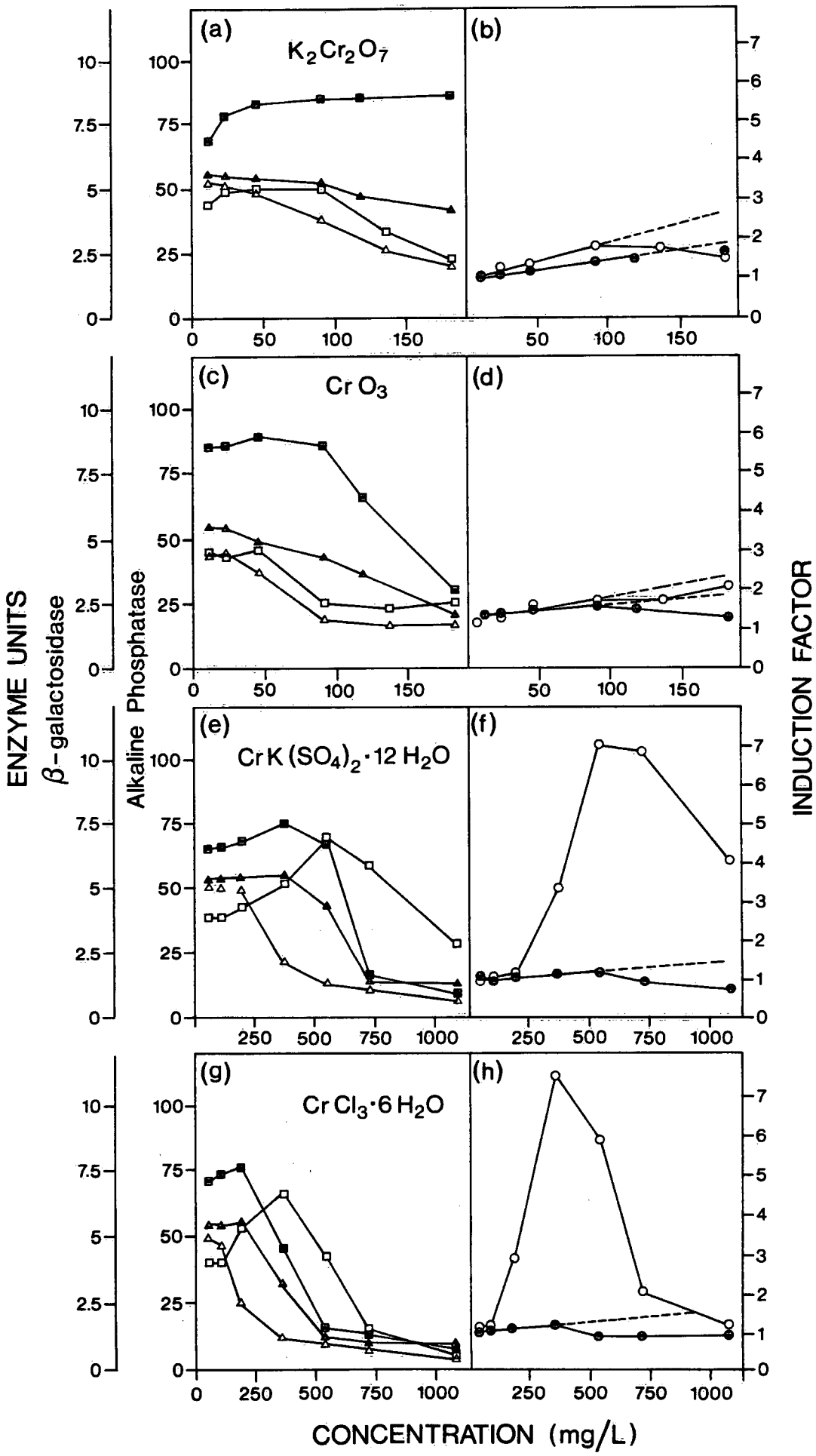
(a)
$K_2Cr_2O_7$
(b)
(c)
CrO_3
(d)
(e)
$CrK(SO_4)_2 \cdot 12H_2O$
(f)
(g)
$CrCl_3 \cdot 6H_2O$
(h)
ENZYME UNITS
β-galactosidase
Alkaline Phosphatase
INDUCTION FACTOR
CONCENTRATION (mg/L)

Fig 2 :

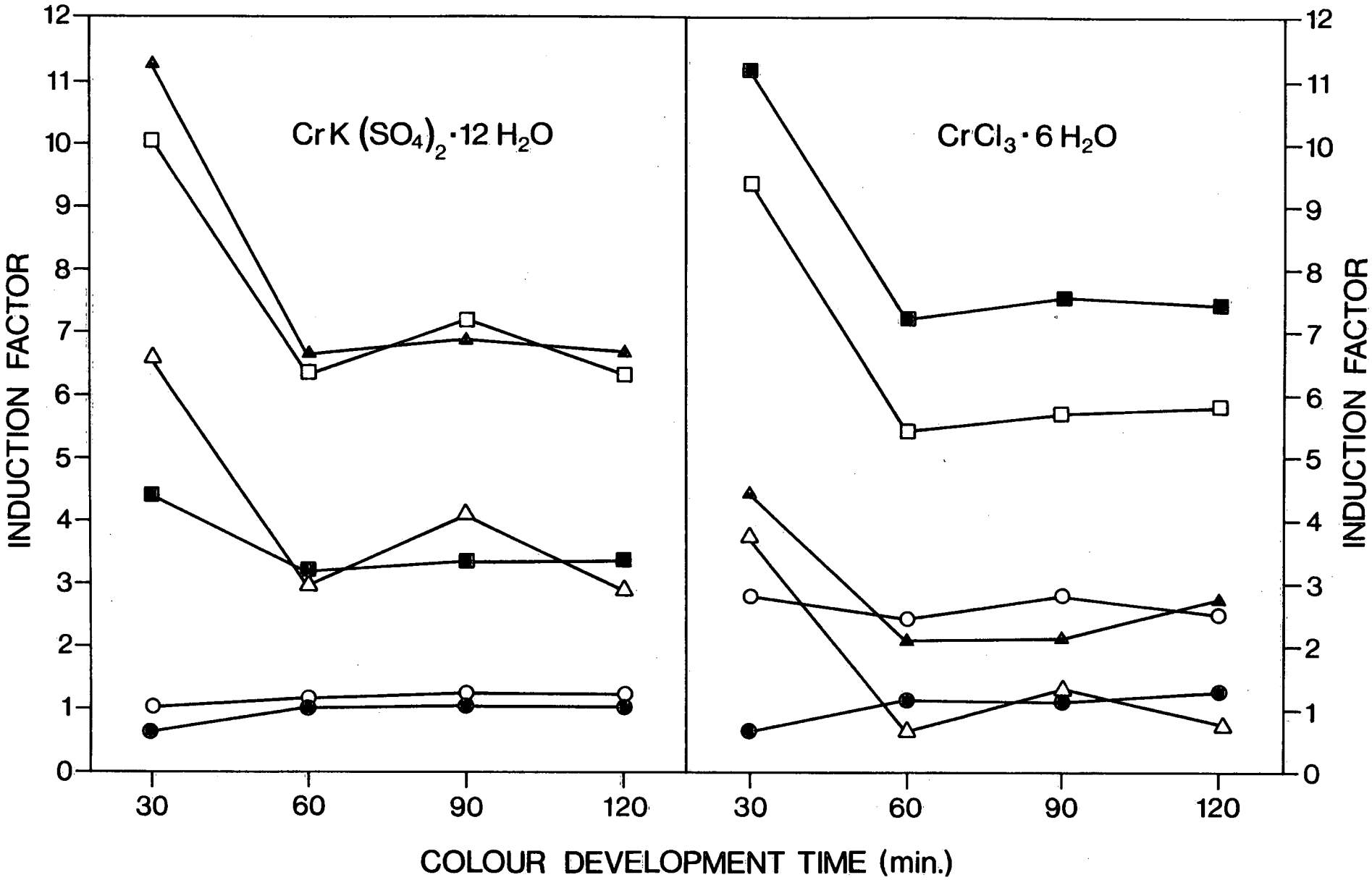

FIGURE 3. VARIATIONS OF CHROMIUM INDUCIBILITIES IN THE SOS CHROMOTEST AT DIFFERENT COLOUR DEVELOPMENT TIME INTERVALS

△—△ 1090 □—□ 545 ○—○ 182

▲—▲ 727 ■—■ 364 ●—● 91

CHEMICAL CONCENTRATION (mg/l)

Figure 4. Variations of quantitative responses obtained with the SOS Chromotest. Dose response curves from $CrK(SO_4)_2.12H_2O$ without S-9 addition were established in three independent experiments. (Expt. 1, open symbols; Expt. 2, half closed symbols; Expt. 3, closed symbols). Chromosomes used in Expt. 3 were from Organics Ltd. SOS Chromotest Kit and are compared to standard non-kit reagents in Expt. 1 and 2. Enzyme units are plotted versus $C_2K(SO_4)_212H_2O$ concentration in (A) (□ ◧ ■ , β-galactosidase units; △ ◭ ▲ alkaline phosphatase units); in (B) induction factor (○ ◐ ●) is plotted versus the concentration.

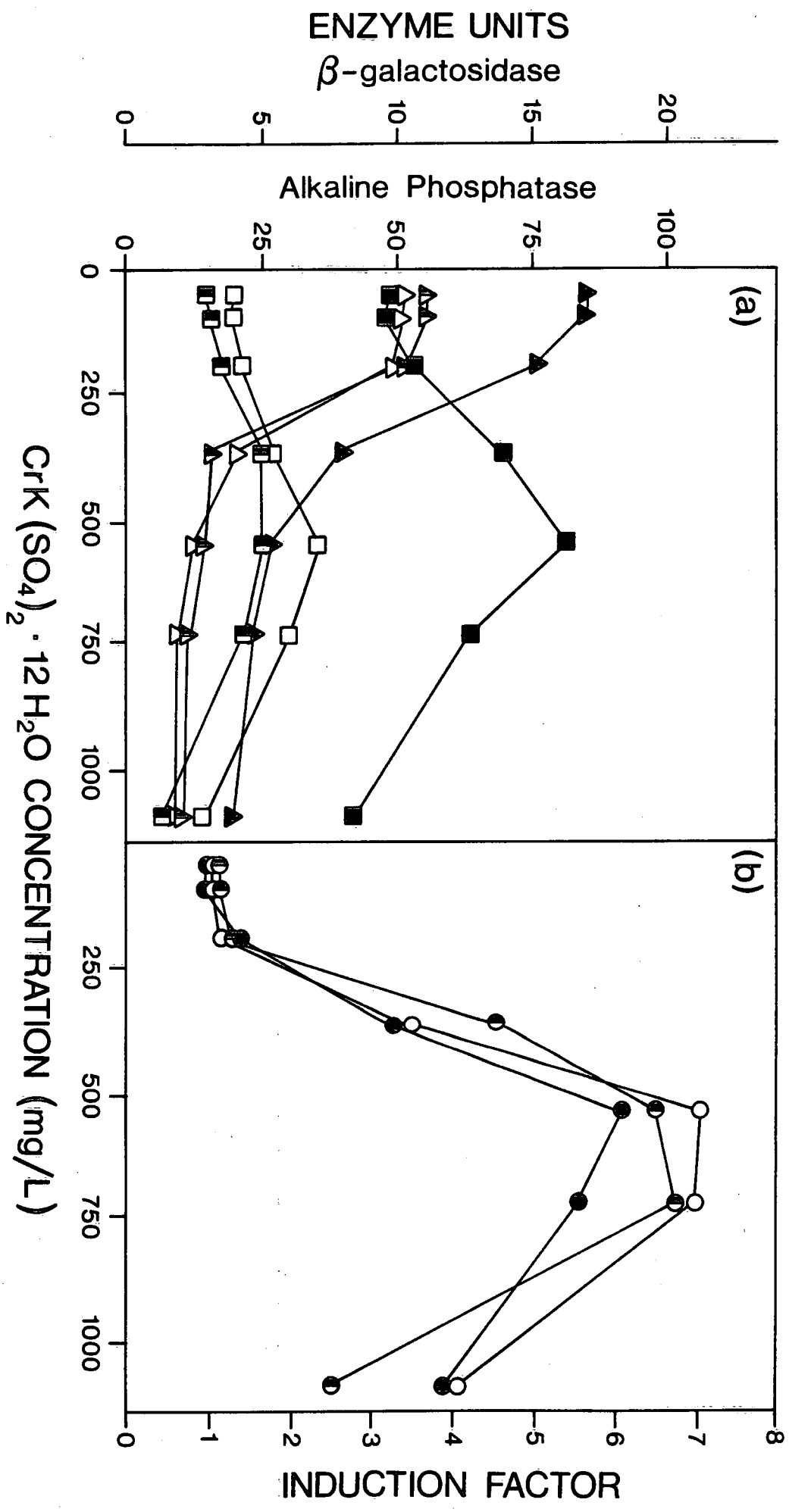

FIGURE 4.

Fig 4

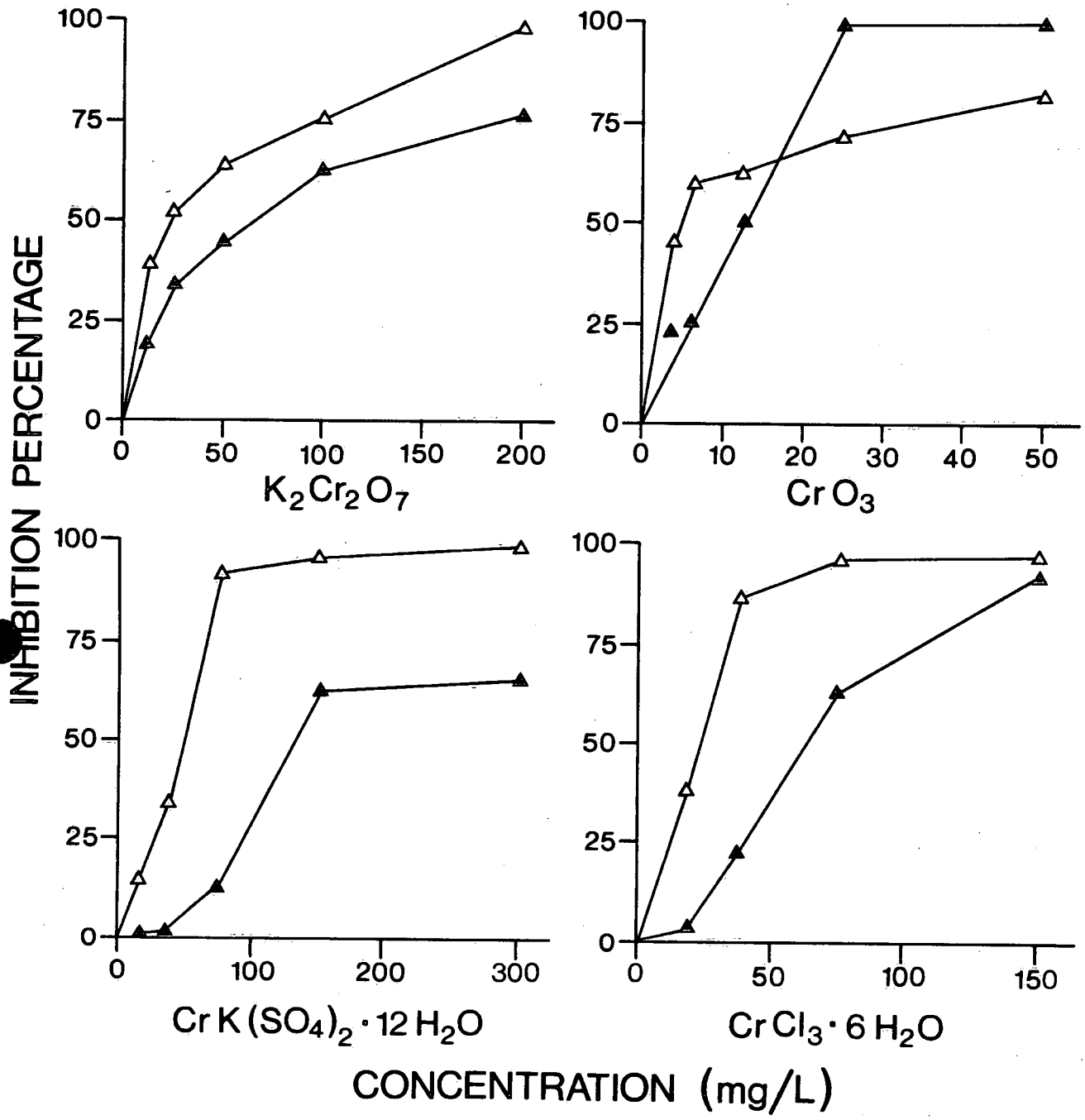

FIGURE 5. COMPARISON OF THE TOXIC EFFECTS OF FOUR CHROMIUM COMPOUNDS AS MEASURED BY THE MICROTOX (▲——▲, 15 min. results) AND ATP-TOX SYSTEM (△——△, 5 hour results).

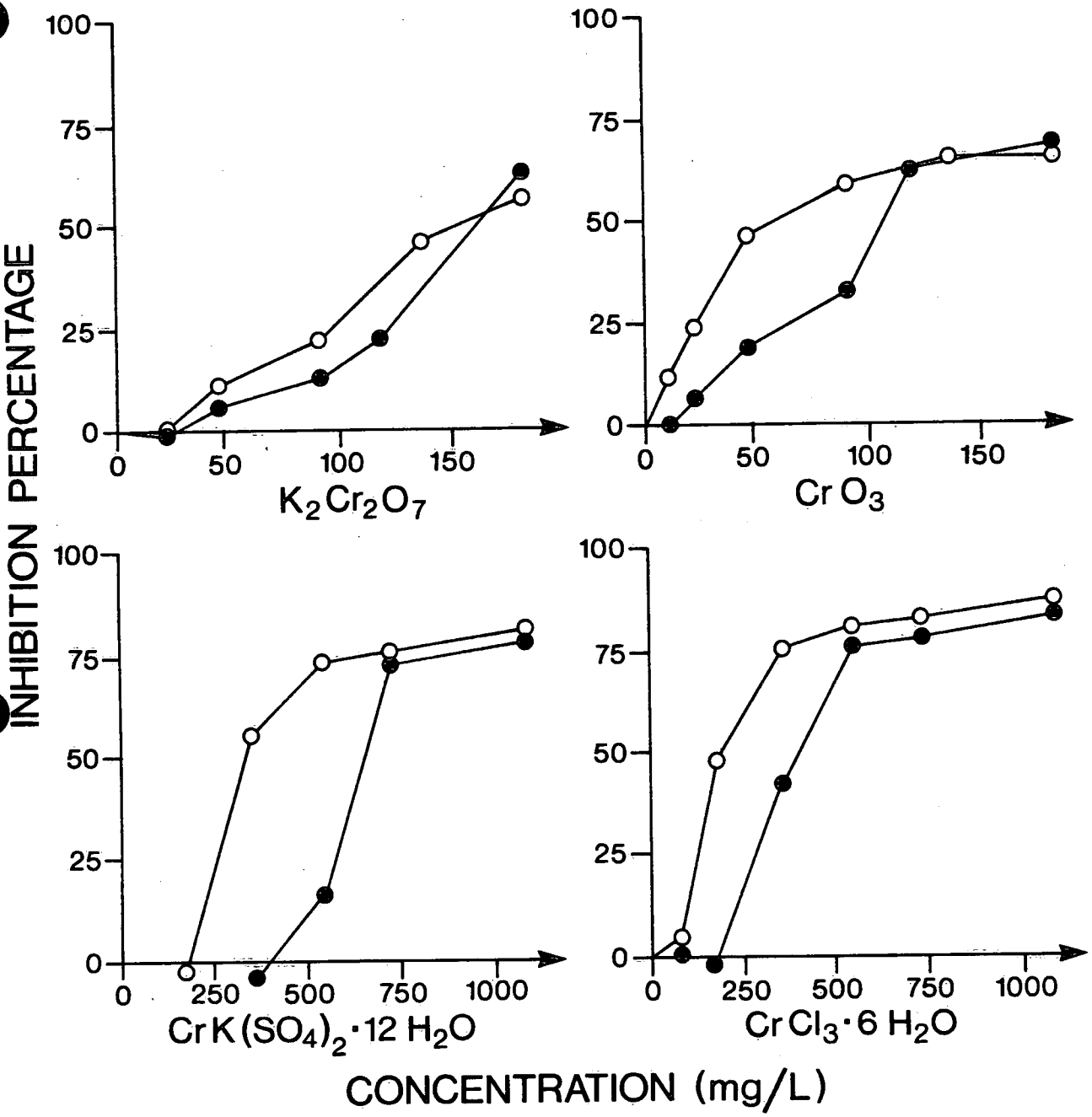

FIGURE 6. INHIBITION OF E.COLI PROTEIN BIOSYNTHESIS BY FOUR CHROMIUM COMPOUNDS AS MEASURED BY ALKALINE PHOSPHATASE ACTIVITY IN THE SOS CHROMOTEST.

○——○ WITHOUT S-9 ADDITION

●——● WITH S-9 ADDITION