Novel Activation Mechanism for the Promutagenic Herbicide Diallate

Abstract. *The potent bacterial mutagen 2-chloroacrolein is formed from the carcinogenic herbicide* S-*2,3-dichloroallyl diisopropylthiocarbamate (diallate) on incubation with hepatic microsomal monooxygenases or on reaction with* m-*chloroperbenzoic acid. A proposed activation mechanism for this promutagen involves sulfoxidation followed by [2,3] sigmatropic rearrangement and 1,2-elimination reactions. A portion of the highly reactive intermediate, diallate sulfoxide (proximate mutagen), is attacked by glutathione in a reaction which competes with its transformation to the ultimate mutagen, 2-chloroacrolein.*

Thiocarbamate herbicides, important chemicals used in producing corn, rice, sugar beets, vegetables, and other crops, are of two types. Most of them are *S*-alkyl or *S*-benzyl compounds, but there are also three commercial *S*-chloroallyl thiocarbamates: diallate or Avadex (*cis*, *trans* mixture) $[(CH_3)_2CH]_2NC(O)SCH_2C(Cl)=CHCl$; triallate or Avadex BW, $[(CH_3)_2CH]_2NC(O)SCH_2C(Cl)=CCl_2$; and CDEC or sulfallate or Vegadex, $(C_2H_5)_2NC(S)SCH_2C(Cl)=CH_2$. Both types of thiocarbamates appear to have similar effects in inhibiting weed growth (*1*), yet there are marked differences between the two groups in other properties, possibly related to the *S*-chloroallyl moiety. The *S*-alkyl and *S*-benzyl thiocarbamates are proherbicides, undergoing metabolic sulfoxidation to form moderately stable sulfoxides that readily carbamoylate thiol compounds (*2*). Sulfoxide metabolites have not been reported for diallate, triallate, and CDEC. The *S*-chloroallyl thiocarbamates, after metabolic activation, are bacterial mutagens, an unfavorable property not shown by the *S*-alkyl and *S*-benzyl thiocarbamates (*3*). Diallate, the most potent promutagen, received the greatest attention in our investigations.

The metabolic conditions under which the promutagenic *S*-chloroallyl thiocarbamates are activated are those likely to yield sulfoxide derivatives. By oxidizing with peracid at temperatures below 0°C, we were able to prepare diallate and triallate sulfoxides and examine their degradation chemistry (Fig. 1). The *S*-3-chloroallyl thiocarbamate sulfoxides undergo a spontaneous [2,3] sigmatropic rearrangement, followed by a 1,2-elimination reaction (*4*). This sulfoxidation, rearrangement, and elimination sequence converts the promutagen diallate to its ultimate mutagen, 2-chloroacrolein.

The *S*-chloroallyl thiocarbamate sulfoxides were prepared by reacting *cis*-diallate, *trans*-diallate, and triallate with equimolar amounts of *m*-chloroperbenzoic acid in methylene chloride at −15°C. They were isolated as crystalline compounds by working at temperatures below 0°C (*5*). These sulfoxides completely decompose at higher temperatures—for example, on thin-layer chromatography (TLC) at 20°C and on holding in chloroform for 10 minutes (*cis*-diallate sulfoxide) or 1 to 2 hours (*trans*-diallate sulfoxide and triallate sulfoxide) at 40°C. Monitoring the degradation rates and identifying the products by nuclear magnetic resonance (NMR) spectroscopy and other methods (*4*) provided an explanation for the instability of the *S*-chloroallyl thiocarbamate sulfoxides in contrast to the analogous *S*-alkyl and *S*-benzyl sulfoxides. *cis*-Diallate sulfoxide, for example, undergoes a rapid and quantitative conversion to 2-chloroacrolein and the carbamoylsulfenyl chloride (Fig. 1). The *trans* isomer gives the same compounds but more slowly. The products originate from a [2,3] sigmatropic rearrangement to a *S*-*O*-allylsulfenate ester followed immediately by a 1,2-elimination reaction (Fig. 1). Triallate sulfoxide degrades by the same mechanism, yielding 2-chloroacrylyl chloride instead of 2-chloroacrolein. The overall sulfoxidation, rearrangement, elimination reaction sequence proceeds much faster with *cis*-diallate than with *trans*-diallate or triallate.

The applicability of these chemical observations to metabolic conditions was examined in vitro and in vivo with mice and rats, using *cis*- and *trans*-diallate labeled with radiocarbon at the carbonyl position in one preparation and at the allyl group in another preparation. Liver microsomes extensively metabolize the diallate isomers, but only when fortified with reduced nicotinamide adenine dinucleotide phosphate (NADPH). Although this suggests sulfoxidation of *cis*- and *trans*-diallate, the sulfoxides were not detected. However, the sulfoxides are very unstable, as pointed out above, and probably react completely within a few seconds or minutes at physiological temperatures. The possible presence of the

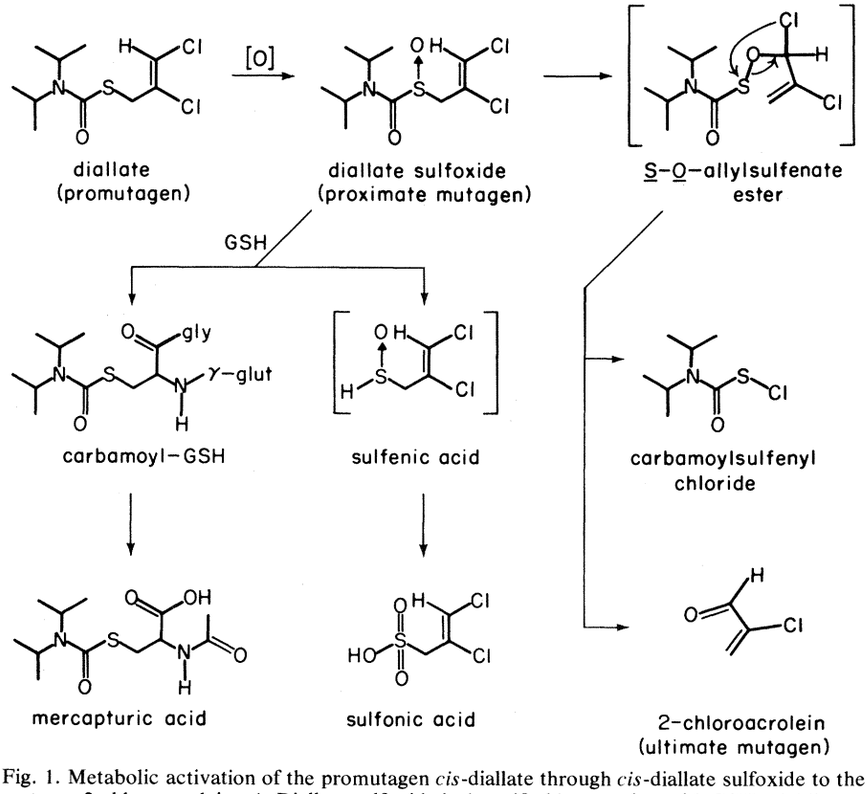

Fig. 1. Metabolic activation of the promutagen *cis*-diallate through *cis*-diallate sulfoxide to the mutagen 2-chloroacrolein. *cis*-Diallate sulfoxide is detoxified by reaction with GSH, yielding as major urinary metabolites the mercapturic and sulfonic acids. Brackets indicate intermediates or compounds probably present but not identified. The same reactions occur with *trans*-diallate, in which the positions of the terminal hydrogen and chlorine are exchanged.

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sulfoxides as transitory intermediates in biological systems was therefore evaluated by identifying other metabolites that would form preferentially or only through the sulfoxides (Fig. 1). In studies with *carbonyl*-^{14}C–labeled *cis*- and *trans*-diallate in mouse microsomal systems (*6*), there was 49 to 67 percent $^{14}CO_2$ without added glutathione (GSH) but only 31 to 40 percent $^{14}CO_2$ when GSH was added; the carbamoyl-GSH derivative accounted for < 1 percent of the radiocarbon in the former case and 28 to 30 percent in the latter case. The diallate isomers do not react with GSH in aqueous acetone, but NMR and TLC studies show that their sulfoxides quickly carbamoylate this important tissue thiol. In the absence of GSH the enzymatically formed [*carbonyl*-^{14}C]diallate sulfoxide undergoes hydrolysis or intramolecular rearrangement reactions, liberating $^{14}CO_2$, whereas with GSH a portion of this intermediate sulfoxide is converted to the relatively stable carbamoyl-GSH derivative. Rats orally given the carbonyl-labeled preparation expired 20 percent $^{14}CO_2$, and their urine contained 62 percent mercapturic acid conjugate (Fig. 1) and 9 percent other metabolites originating from the carbamoyl-GSH derivative (the cysteine and mercaptoacetic acid conjugates). The fate of the chloroallyl portion of the molecule was examined in studies with [*allyl*-^{14}C]diallate. Dichloroallylsulfonic acid, probably formed through the sulfoxide (Fig. 1), was the major metabolite in both mice and rats and their microsome-NADPH systems. Of special importance was the identification of 2-[^{14}C]chloroacrolein in the mouse microsomal oxygenase system (*7*). Analyses by NMR revealed that this aldehyde is not liberated on direct reaction of diallate sulfoxide with GSH in aqueous acetone. These findings establish that under biological conditions diallate sulfoxide undergoes either the major detoxifying GSH conjugation or the minor, competing [2,3] sigmatropic rearrangement reaction followed by 1,2-elimination to liberate a toxicologically significant metabolite.

Mutagenesis studies (Fig. 2) combined with the chemical and biological observations discussed above suggest that the promutagens *cis*- and *trans*-diallate are converted through their sulfoxides (proximate mutagens) to 2-chloroacrolein, the ultimate mutagen (Fig. 1). Diallate is not mutagenic (*8*) unless the microsomal activation system is added, in which case the *trans* isomer gives 25 and the *cis* isomer 40 revertants per nanomole. *cis*-Diallate sulfoxide yields 113 revertants per nanomole, the same value obtained with 2-chloroacrolein (Fig. 2) (*9*); importantly, this is direct mutagenic activity, not dependent on the S9 mix. The greater potency of *cis*- than of *trans*-diallate may be due to more rapid formation of 2-chloroacrolein in the former case.

The fate and mutagenic or herbicidal activity of the diallate isomers appear to depend in large part on formation of the sulfoxides and on their subsequent reactions, which in turn are closely related to tissue GSH levels. The rearrangement and elimination reaction sequence to liberate 2-chloroacrolein occurs only with the portion of the sulfoxide that is not detoxified by reaction with GSH. Herbicidal activity of the diallate isomers may be due to the sulfoxides acting as carbamoylating agents for critical tissue thiols (*2*) or to liberation of 2-chloroacrolein, which is similar in potency to the diallate isomers themselves in inhibiting root growth (*10*).

Triallate sulfoxide, tested immediately after preparation by peracid oxidation, is a potent mutagen (without S9 activation), whereas its decomposition product, 2 chloroacrylyl chloride, is much less active. Since triallate sulfoxide is unstable, it is probably the proximate mutagen. It may be necessary for the chloroacrylyl chloride, which is easily hydrolyzed to the nonmutagenic 2-chloroacrylic acid, to be liberated within the bacteria in order to express its mutagenic activity. Activation of CDEC could involve either initial oxidation at one of the sulfur atoms or hydroxylation at the methylene group adjacent to the sulfur (*11*), the latter reaction leading to spontaneous release of the mutagen, 2-chloroacrolein.

Diallate is carcinogenic in mice (*12*) and CDEC in mice and rats (*13*) but no reports are available on tests with triallate. The findings concerning active mutagenic derivatives from oxidation of *S*-3-chloroallyl thiocarbamate herbicides may have relevance to the mechanism of their carcinogenic activity.

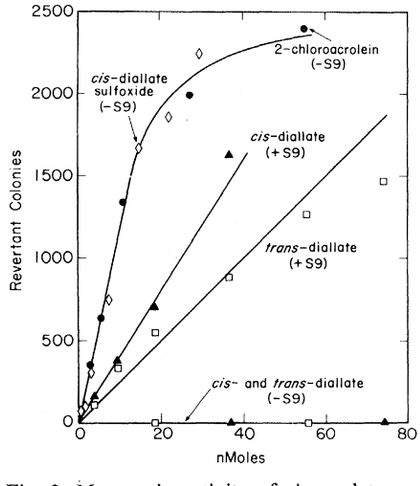

Fig. 2. Mutagenic activity of *cis*- and *trans*-diallate and some of their metabolites, assayed with *Salmonella typhimurium* strain TA 100 sensitive to base-pair substitution mutagens (*14*). The number of spontaneous revertant colonies (~ 120) has been subtracted from the revertant values plotted against amount of mutagen or mixture. Where indicated, S9 mix (containing 20 μl of the 9000*g* supernatant fraction of Arochlor-induced rat liver plus cofactors for xenobiotic metabolism) was incorporated into the top agar. The dose-response relationship is no longer linear at the higher concentrations of 2-chloroacrolein because of accumulation of lethal mutations in the bacteria. 2-Chloroacrolein (− S9) and diallate (+ S9) at 10 to 30 nmole also give significant numbers of revertant colonies with *S. typhimurium* strain TA 98 for detection of frameshift mutagens.

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5. The reaction mixture in methylene chloride at 0° to 5°C was washed with cold 5 percent aqueous sodium carbonate, dried over sodium sulfate, and the solvent evaporated below 0°C by using a vigorous stream of nitrogen.
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7. 2-[^{14}C]Chloroacrolein in the gas phase was trapped in a solution of 2,4-dinitrophenylhydrazine and the resulting hydrazone derivative was isolated, identified by two-dimensional TLC in five different solvent systems, and quantitated by liquid scintillation counting. The yield was < 0.1 percent without NADPH and 1.6 percent with NADPH; that is, the microsomal monooxygenase cofactor is necessary for 2-chloroacrolein formation.
8. Diallate and triallate are not mutagenic even at 135 and 305 nmole per assay plate, respectively (*3*).
9. The mutagenic activity of 2-chloroacrolein on *Salmonella typhimurium* strain TA 100 tested directly or on breakdown of diallate sulfoxide is similar to that of the carcinogen benzo[*a*]pyrene (*14*).
10. 2-Chloroacrolein is approximately equivalent in potency to *cis*- and *trans*-diallate in inhibiting root elongation of oat seedlings. Sulfoxides of the diallate isomers are much less effective, possibly because they decompose on entering the plant without forming 2-chloroacrolein.
11. For an analogous reaction, see Y. S. Chen and J. E. Casida, *J. Agric. Food Chem.* **26**, 263 (1978).

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Visual Resolution and Receptive Field Size: Examination of Two Kinds of Cat Retinal Ganglion Cell

Abstract. *Intraocular recordings from brisk-sustained and brisk-transient ganglion cells in the cat's retina revealed a systematic increase in center size and decrease in spatial cut-off frequency with increasing distance from the area centralis. At any one eccentricity sizes of the centers of sustained and transient cells did not overlap, and the variation in cut-off frequency for each class was constrained to about one-half octave.*

Spatial inhomogeneity within the visual system is reflected in the variation in spatial resolution across the visual field. Thus visual acuity is highest in the fovea and declines progressively as stimulation is moved towards the periphery (*1*). This reduction in spatial resolution is attributed to several factors associated with increasing eccentricity, among them a decline in receptor and ganglion cell density (*2, 3*), a decrease in cortical magnification (*4*), and an increase in receptive field size (*5-7*). We performed experiments to show how both spatial resolution and receptive field size of selected classes of cat retinal ganglion cells vary as a function of eccentricity, and in addition to determine the local variability of these two measures.

The retinal ganglion-cell mosaic is made up of many types of ganglion cells (*6, 7*), which likely subserve different visual functions. It is therefore not sufficient to describe general trends in receptive field properties of ganglion cells taken as a whole; rather, we need knowledge of receptive field properties within a single class as a function of retinal position. This kind of information is most readily obtained through the use of intraocular recording techniques that allow the position of the microelectrode to be varied systematically and accurately. Moreover, it is essential to collect data from as many units as possible within a single retina, for pooling data from several experiments inflates the observed variability.

This report deals only with on-center units with brisk properties (*7*). In all, 180 ganglion cells (138 brisk-sustained units and 42 brisk-transient units) were studied in four cats. In all but seven of these units, we measured, as a function of retinal eccentricity (i) the size of the receptive field center (bar stimuli) and (ii) the spatial cut-off frequency (spatial frequency beyond which there is no significant modulated response) (high-contrast, square-wave gratings). We have specifically avoided examining the unmodulated response observed in brisk-transient units by using fine gratings (*6*).

Experimental methods are essentially the same as those used by Cleland and Levick (*7*). Anesthesia was induced in adult cats by ventilation of 3 to 4 percent halothane in a 2:1 gas mixture of nitrous oxide and carbogen and was maintained during preparatory surgery with 1 to 1.5 percent halothane. To reduce eye movements, the left vagosympathetic trunk was cut and the animal paralyzed by continuous intravenous infusion of an isotonic solution (4 ml/hour) of Flaxedil (5 mg per kilogram of body weight per hour), *d*-tubocurarine (0.4 mg/kg-hour), and glucose. End-tidal P_{CO_2} was monitored and kept at 4 percent by adjusting the stroke volume of the respirator. Bipolar stimulating electrodes (platinum iridium wire in glass) were stereotaxically placed in the left and right optic tracts. The left eye was secured to a micromanipulator by suturing a flap of conjunctiva to a metal ring encircling the globe. The sclera was punctured with a sealed hypodermic needle which allowed the insertion of a tungsten microelectrode (*8*) into the posterior chamber and the precise positioning of the micro-

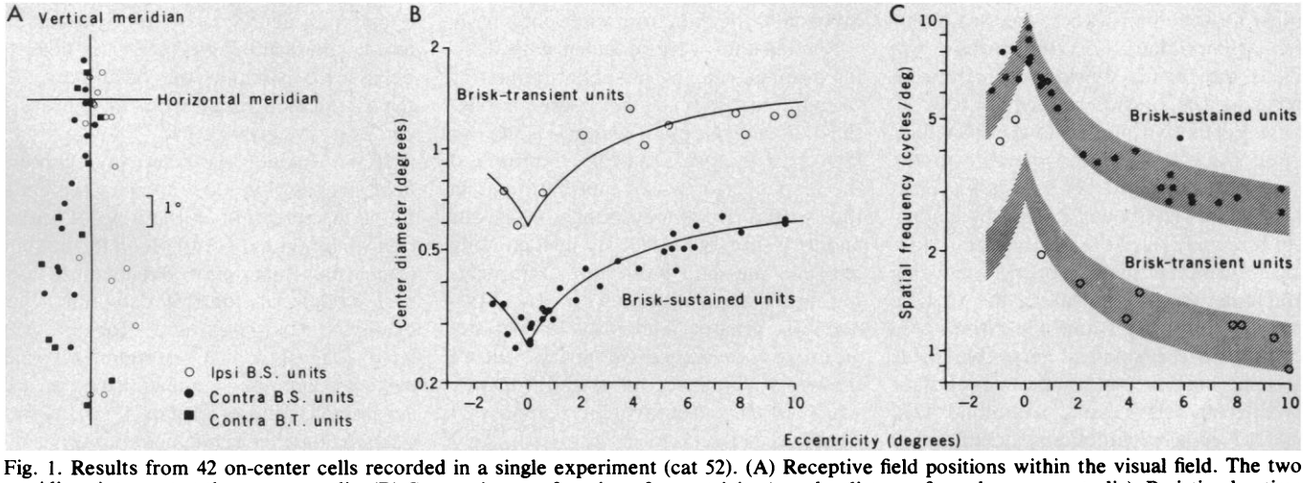

Fig. 1. Results from 42 on-center cells recorded in a single experiment (cat 52). (A) Receptive field positions within the visual field. The two meridians intersect at the area centralis. (B) Center size as a function of eccentricity (angular distance from the area centralis). Peristimulus-time histograms describing the results for a bar (luminance, 25 cd/m², background, 3 cd/m²) moving slowly across the receptive field. Center size was taken as the width of the peak of the histogram at the level of the maintained firing. (C) Spatial cut-off frequency as a function of eccentricity. The shaded area represents a one-quarter octave above and below a hyperbolic curve (reflected) fitted to the sustained units. This area is moved vertically to fit the transient units. Cut-off frequency was estimated from peristimulus-time histograms describing the results of square-wave gratings (mean luminance, 150 cd/m²; contrast, 0.84) moving across the receptive field. Drift frequency, 4 cycles per second.

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