Harmful and Beneficial Effects of Organic Monosulfides, Disulfides, and Polysulfides in Animals and Humans

ARTICLE *in* CHEMICAL RESEARCH IN TOXICOLOGY · JANUARY 2012

Impact Factor: 3.53 · DOI: 10.1021/tx200373u · Source: PubMed

1 AUTHOR:

Rex Munday
AgResearch
141 PUBLICATIONS **3,815** CITATIONS

Chemical Research in Toxicology

REVIEW

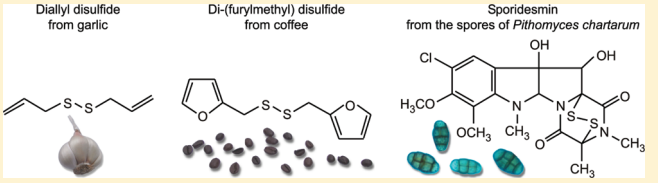
pubs.acs.org/crt

Harmful and Beneficial Effects of Organic Monosulfides, Disulfides, and Polysulfides in Animals and Humans

Rex Munday*

AgResearch, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand

ABSTRACT: Many organic sulfides (mono-, di-, and polysulfides) are present in our environment. Simple derivatives are produced by some plants and animals, while complex sulfides are secondary metabolites of several genera of bacteria and fungi. Sulfides play an important role in the smell and taste of food, and many such compounds are used as food flavorings. Some sulfides are toxic, and there is evidence that such toxicity is caused by the ability of these substances to generate reactive oxygen species. Some sulfides, however, have been shown to protect against toxicants and carcinogens. These beneficial effects are believed to involve, at least in part, the ability of sulfides to inhibit the enzymatic activation of pro-toxicants and to increase tissue activities of enzymes that protect against electrophiles. Some sulfides also have potential as cancer chemotherapeutics. In this review, the toxic and beneficial effects of sulfides in animals are described, and the possible value of sulfides in cancer chemoprotection and cancer chemotherapy is discussed.

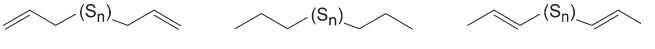


CONTENTS

1. OCCURRENCE AND FUNCTION OF SULFIDES

Organic mono-, di-, and polysulfides (sulfanes) are widely distributed in our environment. Such compounds have a range of industrial uses, and aliphatic and aromatic sulfides are produced as byproducts in industrial processes such as papermaking and oil refining.[1] Sulfides are present in a wide range of human foodstuffs, in which they make a most important contribution to taste and smell. Aliphatic sulfides are formed from certain vegetables, as discussed below, and aromatic and heterocyclic sulfides are formed in foods such as bread, meat and coffee during the cooking or roasting process.[2] Because of the importance of sulfides to the organoleptic properties of food, many synthetic sulfides are used as additives in processed foodstuffs, and more than 80 mono-, di-, and polysulfides are presently listed for use as food flavors.[3]

Plants are a rich source of sulfides. The characteristic taste and smell of vegetables of the *Allium* family, which includes onions, garlic, leeks, shallots, and similar plants, are highly dependent upon these substances. The vegetables contain *S*-alk(en)yl-L-cysteine sulfoxides within vacuoles in the plant cells. When the cells are disrupted by cutting or chewing, the vacuoles are broken, and the cysteine sulfoxides are enzymatically degraded to sulfenic acids, which decompose spontaneously to thiosulfinates. Thiosulfinates are themselves unstable and decay to a complex mixture of compounds, in which mono-, di-, and polysulfides predominate.[4] Garlic and its relatives contain mainly *S*-allyl-L-cysteine sulfoxide, and the major products of decomposition of this substance are diallyl sulfide (diallyl sulfane, DAS, ***1***, $n = 1$), diallyl disulfide (1,2-diallyl disulfane, DADS, ***1***, $n = 2$), diallyl trisulfide (1,3-diallyl trisulfane, DATS, ***1***, $n = 3$), and diallyl tetrasulfide (1,4-diallyl tetrasulfane, DATTS, ***1***, $n = 4$). The dominant compounds in onions and related Alliums are *S*-propyl-L-cysteine sulfoxide and *S*-prop-1-enyl-L-cysteine sulfoxide,[5] and these yield the corresponding dipropyl sulfides DPS, DPDS, DPTS, and DPTTS (***2***, $n = 1-4$) and the diprop-1-enyl sulfides (***3***).

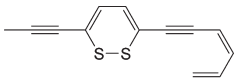


(*1*) (*2*) (*3*)

Received: August 29, 2011
Published: October 17, 2011

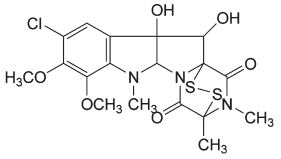
dx.doi.org/10.1021/tx200373u | *Chem. Res. Toxicol.* 2012, 25, 47–60

Plants of the Brassica family (cabbage, Brussels sprouts, cauliflower, kale, etc.) contain *S*-methyl cysteine-L-sulfoxide, from which methyl sulfides are formed on degradation.[6] Flowers of the Titan arum (*Amorphophallus titanum*), the Dead-Horse arum (*Helicodiceros muscivorus*), and succulents of the Stapelia family emit a powerful odor, due to the secretion of dimethyl di-, tri- and tetra-sulfides.[7−9] Plants of the Asteraceae family generate more complex sulfides, such as thiarubrine C (***4***).[10]

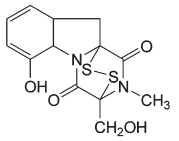


(***4***)

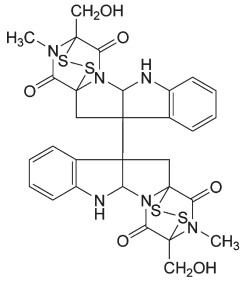
Fungi produce a remarkable range of sulfides, ranging from 1,2,4,6-tetrathiepane (lenthionine) in Shiitake mushrooms[11] to the complex epidithiodioxopiperazine derivatives sporidesmin (***5***), produced by the saprophytic pastoral fungus *Pithomyces chartarum*, gliotoxin (***6***), a secondary metabolite of various fungi of the genus *Ascomycetes*, chaetocin (***7***) and chaetomin (***8***) from *Chaetomium* spp., and acetylapoaranotin (***9***) from *Aspergillus* spp.[12]



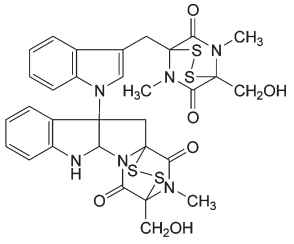
(***5***)



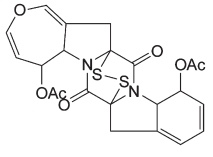
(***6***)



(***7***)

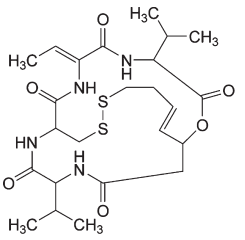


(***8***)

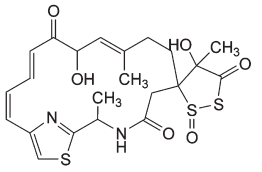


(***9***)

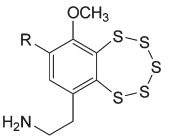
The bacterium *Chromobacterium violaceum* produces the disulfide romidepsin (***10***),[13] while the 1,2-dithiolan-3-one 1-oxide derivative leinamycin (***11***) has been isolated from a Japanese *Streptomyces* species.[14] The pentathiepins varacin (***12***, R = OCH_3) and lissoclinotoxin (***12***, R = OH) have been isolated from ascidians of the *Lissoclinum* genus.[15,16]



(***10***)



(***11***)



(***12***)

Among animals, a notable producer of sulfides is the skunk, whose anal secretion contains predominantly but-2-enyl derivatives.[17]

The function of the sulfides produced by the various organisms is of interest. The powerful taste and smell of the aliphatic sulfides in plants of the cabbage and onion families may act as feeding deterrents against insects and, in some cases, against browsing animals,[5,18] and the foul odor of the skunk secretion acts as a deterrent to predators. The methyl sulfides produced by the arums and stapelias, which are also formed during the decomposition of flesh,[19] are attractive to insects, such as blowflies, that feed or lay their eggs on decaying matter. The advantage to the plants is that the flies pick up pollen when they land on the flowers, and facilitate cross-pollination when visiting other plants of the same species. There is no advantage to the flies, and in the case of stapelias, such mimicry is positively disadvantageous. The appearance and smell of the plants are so similar to rotting meat that flies lay their eggs in the flowers, but on hatching, the larvae inevitably die of starvation. The function of the fungus-derived epidithiodioxopiperazines is not known with certainty, but gliotoxin appears to play a critical role in the virulence of *Aspergillus fumigatus* in animals,[20] and strains of *Pithomyces chartarum* that produce sporidesmin rapidly out-compete strains that lack the ability to synthesize this substance.[21]

2. PRODUCTION OF ROS BY REDOX CYCLING OF SULFIDES

Disulfides are readily reduced to thiols *in vivo* in a thiol−disulfide exchange reaction with reduced glutathione (GSH), catalyzed by glutathione *S*-transferase (GST).[22] Many thiols are unstable and undergo metal-catalyzed oxidation by molecular oxygen to reform the disulfide with concomitant generation of reactive oxygen species (ROS), comprising superoxide radical, hydrogen peroxide, and hydroxyl radical.

The structural requirements for ROS generation from thiols have been reviewed.[1,23] The initial and rate-limiting step of the reaction is electron transfer from the thiolate anion to a transition

dx.doi.org/10.1021/tx200373u |*Chem. Res. Toxicol.* 2012, 25, 47–60

metal in its higher oxidation state, forming the thiyl radical (reaction 1). In the presence of excess thiol, the thiyl radical forms the disulfide radical anion (reaction 2), which rapidly autoxidizes, yielding the superoxide radical and the disulfide (reaction 3). The catalytic cycle is maintained by oxidation of the reduced metal by molecular oxygen (reaction 4). Superoxide formed in reactions 3 and 4 may oxidize more thiol (reaction 5), establishing a radical chain reaction for thiol oxidation. The hydroxyl radical is formed in the metal-catalyzed Haber−Weiss reaction (reaction 6).

$$RS^- + M^{n+} \rightarrow RS^\bullet + M^{(n-1)+} \quad (1)$$

$$RS^\bullet + RS^- \rightarrow RSSR^{\bullet-} \quad (2)$$

$$RSSR^{\bullet-} + O_2 \rightarrow RSSR + O_2^{\bullet-} \quad (3)$$

$$M^{(n-1)+} + O_2 \rightarrow M^{n+} + O_2^{\bullet-} \quad (4)$$

$$RS^- + O_2^{\bullet-} + 2H^+ \rightarrow RS^\bullet + H_2O_2 \quad (5)$$

$$M^{(n-1)+} + H_2O_2 \rightarrow M^{n+} + {}^\bullet OH + OH^- \quad (6)$$

Iron is an effective catalyst for the autoxidation of aliphatic and aromatic thiols, and the oxidation of several such compounds has been shown to be initiated by the reaction of the thiolate anion with the iron moiety of oxyhemoglobin (reaction 7).

$$RS^- + Fe^{II}HbO_2 + 2H^+ \rightarrow RS^\bullet + Fe^{III}Hb + H_2O_2 \quad (7)$$

In view of the involvement of the thiolate anion in initiation of the autoxidation process, it would be expected that only thiols whose pK_a is relatively low will undergo rapid oxidation at physiological pH. Ionization of aromatic and $\alpha\beta$-unsaturated aliphatic thiols is facilitated by charge delocalization, and pK_a values for such compounds are generally less than 7.[24] These compounds will therefore be highly ionized at physiological pH and would be expected to undergo rapid autoxidation under these conditions. The oxidation rates of a number of aromatic thiols have been measured at pH 7, and the relative rates were shown to be in accord with the above considerations.[23] Benzenethiol itself underwent rapid autoxidation, but compounds possessing electron-donating groups (amino, methoxy, and methyl) in the 4-position autoxidized even faster, reflecting increased radical stability.[1] A smaller effect was seen with electron-donating groups in the 2-position, and the rate of autoxidation of benzenethiol derivatives with bulkier groups in the 2-position was very low, reflecting steric hindrance of access of the metal catalyst to the sulfur atom.[1] Substitution with nitro or carboxyl groups led to a marked decrease in oxidation rate, attributable to stabilization of the thiolate anion and destabilization of the thiyl radical by the electron-withdrawing groups.[1] In the presence of excess GSH, aromatic disulfides undergo redox cycling, generating superoxide radical and hydrogen peroxide. The rate of redox cycling was shown to be directly proportional to the rate of oxidation of the thiol formed by reduction of the disulfide.[25]

Complete reduction of a disulfide yields two molar equivalents of thiol, while reduction of a trisulfide would yield an equimolar mixture of thiol and perthiol, while that of a tetrasulfide would generate two molar equivalents of perthiol. The pK_a values of perthiols are significantly lower than those of the corresponding thiols,[26] and these substances undergo rapid autoxidation and redox cycling at neutral pH, again generating ROS.[27,28]

The reduced (dithiol) form of sporidesmin is highly ionized at neutral pH,[29] and the same would be expected of the dithiols formed from other epidithiodioxopiperazine derivatives. Reduced gliotoxin and reduced sporidesmin undergo rapid oxidation at neutral pH, with the generation of superoxide radical, hydrogen peroxide, and hydroxyl radical.[30−32] Redox cycling by gliotoxin and sporidesmin has also been observed.[30,31] In the case of reduced sporidesmin, copper was shown to be a particularly effective catalyst of the autoxidation reaction, while iron had but little catalytic activity.[30] Oxidation of reduced sporidesmin was inhibited by zinc,[33] which forms a stable mercaptide with the thiol groups of this substance.[34]

The pentathiapin ring of varacin and related compounds is reduced by thiols to yield a mixture of polysulfides and perthiols which undergo redox cycling to generate ROS.[35,36] These species contribute to the DNA damage induced by such substances *in vitro* since this was shown to be decreased under anaerobic conditions[35] and ameliorated by metal chelators, catalase, and free radical scavengers.[37] Lienamycin and other 1,2-dithiolan-3-one 1-oxides similarly form perthiols in the presence of thiols,[38,39] with subsequent generation of ROS. Thiarubrine C causes DNA strand scission in the presence of thiols, an effect again attributed to the generation of ROS via redox cycling of the disulfide group.[10] Romidepsin also undergoes redox cycling in the presence of GSH, with the generation of ROS.[40]

ROS formation, with associated oxidative damage, has also been demonstrated in cells incubated with aliphatic and aromatic disulfides, trisulfides, and tetrasulfides, with the effects of these compounds being in direct proportion to their respective rates of redox cycling.[28,41−45] Intracellular generation of ROS has also been demonstrated with leinamycin,[46] romidepsin,[40] sporidesmin,[47] gliotoxin,[31] and chaetomin.[48]

3. INVOLVEMENT OF ROS IN THE TOXICITY OF SULFIDES TO TUMOR CELLS *IN VITRO* AND TO TUMOR XENOGRAFTS *IN VIVO*

The toxic effects of garlic- and onion-derived sulfides on various tumor cell lines *in vitro* have been reviewed.[49−51] These substances suppress cell growth through arrest of the cell cycle in the G2/M phase and by inducing apoptosis. It has been suggested that such changes are initiated by intracellular generation of ROS, leading to inhibition of antiapoptotic factors, induction of pro-apoptotic factors, increased histone acetylation, disruption of microtubules, and activation of caspases 3 and 9. Cytotoxic activity decreases in the order tetrasulfides ≈ trisulfides > disulfides,[43,44,52] which correlates with the order of activity in generating ROS *in vitro*. While the number of sulfur atoms in the molecule is important, the nature of the alk(en)yl group is not since propyl and methyl di- and polysulfides were shown to be as active as their unsaturated counterparts.[53,54] Interestingly, the sulfides are much more toxic to transformed cells than to normal cells, possibly reflecting the greater vulnerability of cancer cells to the harmful effects of ROS.[55,56]

Varacin,[15] leinamycin,[57] romidepsin,[58] gliotoxin,[59] chaetocin,[60] chaetomin,[48] and acetylapoaranotin[61] also induce oxidative stress and apoptosis in cancer cell lines *in vitro*, and the varacin analogue 7-methylbenzopentathiepin is likewise toxic to cancer cells through its ability to generate ROS.[35,36] Diphenyl disulfide, which is reduced to the readily autoxidizable benzenethiol, causes

dx.doi.org/10.1021/tx200373u |*Chem. Res. Toxicol.* 2012, 25, 47–60

apoptosis of leukemia cells *in vitro*.[62] Benzenethiol derivatives are similarly toxic to leukemia cells, with compounds possessing electron-donating substituents in the ring being more active than those with electron-withdrawing groups,[63] consistent with the effects of such substituents on the rate of ROS generation *in vitro*.

DATS and DADS have also been shown to suppress the growth of many tumor xenografts in athymic mice,[49,50] as has the prop-1-enyl derivative 2-(prop-1-enyl[thiosulfinyl])acetic acid.[64] As in the case of isolated cells *in vitro*, changes in the degree of histone acetylation and expression of proteins modulating apoptosis, together with apoptosis induction, have been observed in the transplanted cells.[65−68]

Bis-(4-fluorobenzyl) trisulfide inhibited the growth of xenografts of human cancer cell lines in athymic mice.[69] Gliotoxin was toxic to xenografts of human tumors in immunocompromised mice[70] and inhibited the growth of mammary tumors induced by *N*-methyl-*N*-nitrosourea in rats.[71] Chaetocin, chaetomin, and acetylapoaranotin were shown to suppress the proliferation of myeloma cells in immunocompromised animals[60] and of hepatoma, colon, and prostate cancer cell xenografts in athymic mice.[61,72,73]

On the basis of these results, it has been suggested that allyl sulfides, particularly DATS, could be valuable in cancer therapy.[50,52,74] It has also been suggested that gliotoxin, chaetocin, and chaetomin could find use as anticancer agents in humans.[48,60]

4. INVOLVEMENT OF ROS IN THE TOXICITY OF SULFIDES TO ANIMALS

Interest in the *in vivo* toxicity of sulfides was stimulated by observations that garlic and onions cause oxidative hemolysis, leading to anemia, when ingested by domestic and laboratory animals. Such hemolysis is caused by the generation of oxidizing species within erythrocytes, leading initially to oxidation of oxyhemoglobin to methemoglobin. Further oxidation results in the formation of irreversibly damaged hemoglobin, which precipitates within the cells and adheres to the cell membrane, where it forms spherical clumps recognized as Heinz bodies.[75] The presence of Heinz bodies within erythrocytes causes a decrease in cellular deformability,[76] and such cells may lyse within the circulation, leading to hemoglobinemia. Oxidatively damaged erythrocytes are also trapped and destroyed within splenic sinusoids.[77] In rodents, the increased rate of destruction of red blood cells leads to compensatory erythropoiesis in the spleen, liver, and kidneys,[78] but if the rate of erythrocyte destruction exceeds the rate of compensatory erythropoiesis, the animal becomes anemic. Splenic enlargement is a prominent pathological finding in oxidative hemolysis, due to proliferation of erythropoietic cells of the red pulp.[79] This organ may also become unusually dark in color, reflecting storage of iron in the form of hemosiderin, derived from lysed erythrocytes.[78] When cells are lysed within the bloodstream (intravascular hemolysis), the released hemoglobin is excreted via the kidney, and in severe cases, hemoglobin and its degradation products are taken up by tubular epithelial cells, causing hemoglobinuric nephrosis.[80] Such renal damage, or severe anemia, may result in death.

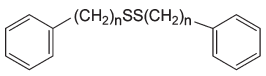
Anemia induced by garlic and onions has been observed in dogs,[81] cats,[81] cattle,[80] sheep,[82] horses,[83] pigs,[84] goats,[85] rabbits,[85] geese,[86] chickens,[87] rats,[88] and mice.[89] In all cases, the characteristic signs of oxidative hemolysis were observed. *Allium*-induced hemolysis is most frequently seen in cattle, cats, and dogs, and the effects are often very severe, with intravascular hemolysis regularly occurring in these animals. In cattle, death is often observed. In these animals, the severe toxicity may simply reflect the amount of vegetable matter eaten: cows may consume up to 20 kg of onions per day.[90] In contrast, cats and dogs appear to be intrinsically vulnerable to oxidative hemolysis and may suffer anemia with very low intakes of onion. This was well illustrated by findings in cats in the 1980s. Sick or debilitated cats are sometimes fed meat-based baby foods, which they find highly palatable. In 1985, the manufacturer of one popular brand of baby food began adding small amounts of onion powder to their product. Soon afterward, there were reports of Heinz body hemolytic anemia in cats fed the new formulation,[91] and later studies proved that the onion powder was the cause of the problem.[92] In all species, onions are more potent hemolytic agents than garlic.[81] Humans appear to be relatively resistant to the toxic effects of *Allium* vegetables. No adverse effects of garlic were reported in individuals eating 10−15 g of raw garlic per day for up to 2 months.[93,94]

The observations in animals led to investigation of the vegetable-derived substances responsible for the hemolytic effect. In 1931, Gruhzit[95] showed that dipropyl disulfide caused hemolysis in dogs and attributed the hemolytic action of onions to this substance. The involvement of lipophilic materials derived from onions was confirmed by the observation that an ether extract of macerated onion was strongly hemolytic in cats.[96] Later work has shown that while dipropyl disulfide may contribute to the hemolytic action of onion, diprop-1-enyl disulfide is a much stronger hemolytic agent.[97] Furthermore, DADS from garlic is less potent than diprop-1-enyl disulfide from onions, which could account for the fact that the hemolytic activity of garlic is less than that of onions.[97] In a comparative study of allyl and propyl mono-, di-, tri-, and tetra-sulfides, the hemolytic activity was in direct proportion to rates of redox cycling *in vitro*.[28]

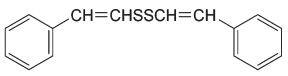
Excessive consumption of Brassica vegetables also leads to oxidative hemolysis (kale anemia) in ruminant animals. In this case, the hemolytic effect is attributable to methyl sulfides.[6] Another interesting case of hemolytic anemia attributed to sulfides was that seen in a dog that had been sprayed by a skunk.[98]

Certain aromatic disulfides also cause oxidative hemolysis in animals,[41,99] with activity again correlating with the rate of redox cycling and the rate of autoxidation of the thiol formed by reduction. The correlation was so consistent with these substances and with aliphatic sulfides that it was suggested that hemolytic activity could be predicted on the basis of the rate of redox cycling promoted by oxyhemoglobin *in vitro*.[100] Later studies have shown, however, that this is only partly true. Diprop-1-enyl sulfide, which, being a monosulfide, cannot directly undergo redox cycling and showed no effects *in vitro*, but it caused hemolysis in rats.[101] It was suggested[101] that this effect is due to *in vivo* metabolism of the sulfide to prop-1-ene-1-thiol, which is the reduction product of the strongly hemolytic diprop-1-enyl disulfide. Furthermore, a comparative study with bis(phenylmethyl), bis(phenylethyl), and bis(phenylpropyl) disulfides (***13***, n = 1, 2, and 3, respectively) gave surprising results. *In vivo*, these compounds are reduced to phenylmethanethiol, phenylethanethiol, and phenylpropanethiol, respectively, all of which are less than 0.5% ionized at neutral pH and would therefore not be expected to undergo rapid autoxidation. In accord with this expectation, none of the disulfides underwent redox cycling *in vitro* and would thus have been expected to be of low hemolytic activity. This was found to be true for bis(phenylmethyl) disulfide and bis(phenylpropyl) disulfide, which showed no hemolytic activity in rats, but bis(phenylethyl) disulfide was a powerful hemolytic agent. It was suggested[102] that this effect is attributable to *in vivo* β-oxidation of phenylethanethiol

to phenylethenethiol, an $\alpha\beta$-unsaturated thiol that would be expected to undergo rapid autoxidation. Bis(phenylethenyl) disulfide (**14**) proved to be a potent hemolytic agent in rats.[102] Therefore, while some hemolytic sulfides may be recognized by their reactivity toward oxyhemoglobin *in vitro*, the possibility of metabolic activation *in vivo* must always be borne in mind.



(**13**)



(**14**)

The target site of epidithiodioxopiperazines is not erythrocytes but biliary epithelial cells. Ruminant animals grazing pasture contaminated with *Pithomyces chartarum* suffer the hepatogenous photosensitization disease facial eczema due to the mycotoxin sporidesmin. This disease is a particular problem in New Zealand because of the climate, local farming practices, and the fact that most strains of *Pithomyces* in this country produce sporidesmin. It is responsible for losses in animal production of up to $120 million/year.[103] After ingestion, sporidesmin is rapidly absorbed and excreted via the biliary system. It becomes concentrated as it passes down the biliary tree[104] and causes necrosis of the bile duct epithelium. Granulation tissue subsequently invades the disorganized area around the bile ducts, and in severe cases, the major bile ducts become totally obstructed.[105] This leads to the retention of phylloerythrin, a breakdown product of chlorophyll, which is normally excreted in bile. Phylloerythrin is a photosensitizer, and when exposed to sunlight, animals develop the skin lesions that are the characteristic clinical signs of the disease. The same lesions are provoked in experimental animals dosed with sporidesmin[21] or with gliotoxin.[106] The susceptibility of the biliary epithelium to sporidesmin-induced damage is attributable to the concentration of the toxin in bile and to the high sensitivity of biliary epithelial cells toward oxidative damage.[107]

Zinc salts afford protection against facial eczema, and administration of zinc is now a routine practice in New Zealand for protecting against sporidesmin toxicity.[108] The protection given is believed to reflect the inhibition of ROS formation from sporidesmin through complex formation between zinc and reduced sporidesmin, and through diminution by zinc of the transport pool of copper, thereby depleting tissue levels of this metal required for the catalysis of the autoxidation of reduced sporidesmin.[30] This hypothesis is supported by the fact that iron salts, which, like zinc, inhibit copper uptake from the gastrointestinal tract, afforded protection against facial eczema,[109] although the effect of iron was much lower than that of zinc, suggesting that the most important mechanism of zinc prophylaxis involves complex formation. The importance of ROS in facial eczema is supported by the observation that antioxidants protect against sporidesmin toxicity to sheep.[110] No information on the toxicity to animals of other epidithiodioxopiperazines is available.

5. EFFECT OF SULFIDES ON PHASE 1 ENZYME ACTIVITY

The effects of sulfides on the activities of phase 1 enzymes in animals following the administration of single doses of the test compounds have been reviewed.[111]

DAS, DADS, and DATS decreased the expression and activity of hepatic P450 2E1, while dimethyl sulfide and dimethyl disulfide were without effect. Propyl sulfides were reported to have either no effect on P450 2E1 or to increase its activity. Clearly, the presence of an alkene moiety is necessary for the inhibition of this enzyme in the liver. In the case of DAS, active metabolites play a significant role in the inhibitory process. *In vivo*, DAS undergoes oxidation at the sulfur atom to form the corresponding sulfoxide and sulfone. Both the parent compound and the sulfoxide are competitive inhibitors of P450 2E1, while the sulfone is a suicide inhibitor, an effect that has been attributed to the formation of an epoxide across one of the allyl groups to form 2-[(allylsulfonyl)methyl]oxirane.[112] Whether the inhibition of P450 2E1 by DADS and DATS similarly involves metabolites is not known, and the effect of prop-1-enyl derivatives on this enzyme has not been investigated.

DAS and DPS are potent inducers of enzymes of the P450 1A and P450 2B subfamilies in rodent liver. DADS, DATS, DPDS, and dimethyl disulfide also increased the activity of these enzymes but with lower efficacy.[111] Induction of these enzymes is thus not dependent upon the presence of an allyl group, although the mechanism of induction is not known.

Neither DAS nor DADS influenced the activity of P450 2C11, and no significant effect of these compounds or of DATS, DPS, or DPDS were observed on the activities of P450 3A1 or P450 3A2.[111]

In all the reported studies, very high levels of the sulfides have been employed, and there is little available information on the relationship between dose and effects on phase 1 enzymes. In the case of DAS, P450 1A1 activity was increased, and P450 2E1 was decreased in rat liver at a dose of 175 μmol/kg, but no significant effect on these enzymes was seen at a dose of 88 μmol/kg.[113]

Although attention has been focused on the effects of the sulfides on hepatic levels of the phase 1 enzymes, some data are available on effects in other tissues. DAS is reported to decrease the activity of P450 2E1 in the kidney and lung of rats,[114] although no effect of this compound or of DADS was observed in the intestine.[115] DAS increased the activity of P450 1A1 and P450 2B1 in the duodenum and stomach of rats,[116,117] but neither this compound nor DADS or DATS showed any effect in the lung or jejunum.[118] In mice, DAS was reported to cause a slight, but statistically significant, decrease in pulmonary P450 1A1.[119]

Garlic oil, which contains a mixture of sulfides, mainly allyl derivatives, is reported to cause a significant decrease in P450 2E1 activity in humans.[120] This result is surprising since the total intake of sulfides by the participants in this study was only ~0.03 mg/kg/day. In animals, doses 3 orders of magnitude higher are required to produce significant inhibition of P450 2E1. Furthermore, while the effects of sulfides on enzymes of the P450 1A subfamily were shown to be much greater than those on P450 2E1 in animals, no effect on the activity of P450 1A2 was seen in humans.[120] In another experiment (unpublished), cited by Wilkinson,[121] no effect on P450 2E1 activity was seen in humans with garlic or with high doses of garlic products.

6. EFFECT OF SULFIDES ON PHASE 2 ENZYME ACTIVITY

The phase 2 enzymes, which include glutathione *S*-transferase (GST), NAD(P)H:quinone acceptor oxidoreductase (NQO1), uridine diphosphate glucuronosyltransferase (UGT), and epoxide hydrolase (EH), detoxify electrophilic toxins, converting them to water-soluble substances that can readily be excreted.[122] Numerous animal studies have shown that an increase in tissue levels of phase 2 enzymes affords protection against chemically

dx.doi.org/10.1021/tx200373u |*Chem. Res. Toxicol.* 2012, 25, 47–60

induced toxicity,[123,124] while certain *GST* and *NQO1* null genotypes are associated with increased risk of cancer in humans.[125]

The literature up to mid-2003 on the effects of sulfides derived from onions and garlic on tissue levels of phase 2 enzymes has been reviewed and structure activity relationships discussed.[126] The importance of unsaturation and of the number of sulfur atoms in the molecule was stressed, and more recent studies[28,101,127] have confirmed these conclusions. In the allyl series, inductive activity in rat liver decreased in the order DATTS ≈ DATS > DADS > DAS. In the prop-1-enyl series, however, the reverse order of activity was recorded, with diprop-1-enyl sulfide being a stronger inducer than diprop-1-enyl disulfide. In the propyl series, the number of sulfur atoms had no significant effect on inductive activity, with DPTTS, DPTS, DPDS, and DPS all showing little or no effect on hepatic phase 2 enzyme activity.[28,101] Dimethyl, diethyl, and dibutyl trisulfide were similarly of very low inductive activity.[127]

Although the focus of many studies on the effect of sulfides on hepatic phase 2 enzyme activities has been the liver, enzyme induction, often of greater magnitude than that seen in the liver, has been observed in other tissues. DAS was a relatively weak inducer in all the tissues examined, as were all the propyl derivatives. Diprop-1-enyl sulfide showed high activity in the spleen, forestomach, glandular stomach, duodenum, jejunum, ileum, cecum, and colon of rats, while DADS, DATS, and DATTS had pronounced effects in the spleen, kidney, lungs, forestomach, and glandular stomach, and throughout the small and large intestines.[28,101,126] None of the compounds except diprop-1-enyl sulfide showed marked effects in the urinary bladder, and none was particularly effective in the heart. Dose–response experiments with DADS and diprop-1-enyl sulfide confirmed the tissue selectivity of enzyme induction. DADS showed the greatest effects in the forestomach and duodenum, causing statistically significant increases in phase 2 enzyme activity in these tissues at only 2 μmol/kg/day.[131] Diprop-1-enyl sulfide was somewhat less effective in the duodenum, with a dose of 10 μmol/kg/day being required for increased phase 2 enzymes activity, but this compound was more effective than DADS in the large intestine, with significant increases again being observed at 10 μmol/kg/day.[101]

The duration of dosing has a marked effect on the degree of enzyme induction provoked by sulfides. Rats dosed with DAS or DADS each day for 13 or 15 days, or with DAS 3 times per week for 7 weeks, showed greater effects on hepatic EH, UGT, and/or GST activities than animals receiving similar doses over a shorter period of time.[115,128,129] In a long-term experiment,[130] rats were fed diallyl disulfide at a level of 200 ppm in the diet for a total of 56 weeks. After this time, hepatic GST, NQO1, and UGT activities were increased by factors of 3.8, 1.5, and 1.8, and GST and NQO1 activities were increased 3.3- and 1.5-fold in the colon. This dietary level of diallyl disulfide equates to a daily dose of only 68 μmol/kg. In short-term experiments, this degree of enzyme induction is achieved only at dose levels of ~600 μmol/kg,[131] indicating that long-term administration of sulfides may lead to cumulative increases in phase 2 enzyme activity.

The mechanism whereby sulfides increase tissue levels of phase 2 enzymes has not been definitively established. A major mechanism of such enzyme induction by various chemical compounds involves activation of the transcription factor nuclear factor E2-related protein 2 (Nrf2).[132] Nrf2 is normally bound by its repressor, Kelch-like ECH-associated protein 1 (Keap1), and targeted for degradation by the ubiquitin–proteosome system. Inducers of phase 2 enzymes have been shown to disrupt the Nrf2-Keap1 complex, preventing the degradation of Nrf2. Cytoplasmic concentrations of this factor then increase, and Nrf2 migrates to the nucleus, binding to the antioxidant response element in the promoter region of phase 2 genes, thereby stimulating gene transcription. While the nature of inducer–Keap1 interactions is not fully understood, there is evidence that modification of thiol groups on Keap1, through oxidation, thiocarbamoylation, or alkylation, may play an important role in disrupting the complex.[124,133] The involvement of ROS in this process has been suggested.[124,134]

The induction of phase 2 enzymes by allyl sulfides is also mediated by Nrf2,[135,136] although the nature of the interaction with Keap1 has not been identified. It is unlikely to involve ROS since although DPTS and DPTTS are powerful oxidants, they have little or no effect on phase 2 enzyme activity. Only allyl and prop-1-enyl derivatives are effective in increasing tissue enzyme activities, and the role of the unsaturated linkage requires further investigation.

7. CANCER CHEMOPREVENTION BY SULFIDES

Interest in the possible anticancer activity of *Allium*-derived sulfides was stimulated by reports of a highly significant inverse relationship between the intake of *Allium* vegetables (garlic, scallions, Chinese chives, and onion) and incidence of stomach cancer in northern China.[137] Subsequent epidemiological studies up to 2001 have been reviewed,[138] with the conclusion that there is a positive correlation between the intake of *Allium* vegetables and decreased risk of gastric and colorectal cancer. The same conclusion was drawn in the 2007 report of the World Cancer Research Fund/American Institute for Cancer Research.[139] While an analysis using the evidence-based review system of the United States Food and Drug Administration showed that there was insufficient evidence for a health claim regarding garlic,[140] a recent meta-analysis again showed a reduction in gastric cancer incidence with high consumption of *Allium* vegetables.[141] Two intervention studies have been reported, both conducted in China. In the first, DATS, together with low doses of sodium selenite, were dosed to a total of 5033 individuals every other day for one month each year for 2 years.[142] After a follow-up period of 10 years, 23 cases of gastric cancer out of 2526 participants were seen in the test group, compared with 30/2507 in the control group, a nonsignificant difference.[143] The intermittent and short-term administration of DATS did not, however, relate to the normal use of garlic, which in some parts of China is eaten daily at up to 20 g/day throughout life.[144] A second intervention trial, in which participants received 200 mg of aged garlic extract and 1 mg of garlic oil, twice daily for 7.3 years, showed no beneficial effects on the prevalence of precancerous gastric lesions or on gastric cancer incidence.[143] Again, this trial did not fully replicate garlic intake since only microgram amounts of sulfides would be derived from 1 mg of commercial garlic oil, and aged garlic extract contains mainly *S*-allylcysteine and *S*-allylmercaptocysteine,[145] which are present only in trace amounts in whole or crushed garlic cloves.[146]

In animal experiments on the possible protective effects of *Allium*-derived sulfides, much attention has unfortunately been focused on DAS in the mistaken belief that this substance is a major product of the decomposition of garlic sulfoxides. It is also unfortunate that in the majority of reported experiments, DADS was purchased from Aldrich. This product contains only 80% DADS. The remaining 20% is stated to be "other sulfides", the properties of which could confound the conclusions drawn from

experiments with this preparation of DADS. In future experiments, it would be preferable to employ pure DADS. This substance is easily synthesized.[126]

Many test protocols have been employed, in which the sulfide has been administered before, with, or after the carcinogen, or, most commonly, both before and after carcinogen administration. Very high dose-levels have been employed, and the sulfides have been administered by various routes. In some experiments, the effect of the carcinogen was potentiated by partial hepatectomy, by concurrent induction of hepatic necrosis, or by coadministration of promoters. Efficacy has been determined by incidence and/or multiplicity of tumors, or, more frequently, by examination of preneoplastic lesions such as induction of the placental form of GST or ornithine decarboxylase, production of nuclear aberrations or DNA single-strand breaks, or formation of DNA adducts.

DAS, DADS, DATS, and diprop-1-enyl sulfide, administered before the carcinogen, protected against the skin cancer induced by multiple applications of polycyclic hydrocarbons.[147−149] Multiple doses of DADS and DATS, again given before the carcinogen, suppressed the induction of forestomach and mammary cancer by polycyclic hydrocarbons. DAS gave less protection, and propyl sulfides were without significant effect.[150,151]

DADS, fed to rats both before and after the administration of the carcinogen, was more effective than DAS in decreasing the incidence of hepatic preneoplastic lesions induced by aflatoxin B_1 (AFB_1).[152,153] Both DAS and DADS were reported to decrease the incidence of colonic preneoplastic changes induced by azoxymethane (AOM).[154] DAS, given as a single dose shortly before the administration of 1,2-dimethylhydrazine (DMH) decreased the incidence of preneoplastic changes in the colon of rats,[155,156] but DADS, DPS and DPDS were without effect.[155] DAS had no significant effect on colonic lesions in mice dosed with *N*-methyl-*N*-nitrosourea (MNU) or *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG),[156] although a single dose of DAS given shortly before the carcinogen decreased the incidence of preneoplastic changes in the glandular stomach of rats dosed with the latter substance.[157] DADS, given in the diet for 14 days before exposure to the carcinogen, decreased the incidence of mammary tumors in rats receiving MNU.[158]

DAS, DADS, and DPDS, when given before the carcinogen once a week for 8 weeks, decreased the incidence and/or multiplicity of papillomas and carcinomas in the forestomach of animals receiving *N*-nitrosodiethylamine (NDEA). DAS and DADS also decreased the incidence of pulmonary tumors induced by this substance.[159] DAS and DADS, fed in the diet of rats for 2 weeks before the administration of the carcinogen, decreased the incidence of preneoplastic changes in the livers of rats dosed with *N*-nitrosodimethylamine (NMDA).[153] Multiple doses of DAS decreased the incidence and multiplicity of esophageal tumors provoked by *N*-nitrosomethylbenzylamine (NMBA) when given before the carcinogen but not when given afterward.[160]

Repeated doses of DAS, given before the carcinogen, decreased the incidence and multiplicity of pulmonary tumors induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)[161] and the incidence of preneoplastic lesions in the colon of rats dosed with 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine (PhIP).[162] DADS, given before and during exposure to PhIP, decreased the incidence and multiplicity of mammary tumors.[163] DAS decreased the multiplicity of skin papillomas induced by vinyl carbamate when given as a single dose shortly before the carcinogen,[164] while multiple doses decreased the incidence of preneoplastic lesions in the liver of rats dosed with 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ).[165]

DAS decreased the incidence of nuclear aberrations induced by cyclophosphamide in the urinary bladder of mice when given as a single dose 30 min before the toxin,[166] and DATS decreased the formation of hepatic DNA adducts by acrylamide when dosed daily to mice for 6 days before the carcinogen.[167]

While most studies have shown a protective effect of sulfides against carcinogens, some have demonstrated an adverse effect. DAS, when given postinitiation, was reported to increase the incidence of preneoplastic changes induced by NDEA in rats, although DADS and DPDS had no significant effect.[168,169] DAS was also shown to increase the incidence of preneoplastic changes in the colon of rats dosed with AOM.[170]

8. PROTECTION AGAINST TOXIC CHEMICALS BY SULFIDES

Diallyl sulfone protected against the hepatotoxicity of acetaminophen when given as a single dose shortly before the toxin but had little or no effect when given afterward. DAS was much less effective,[171] and under the same conditions, DADS and DPS had no significant effect.[172] DAS protected against the hepatotoxicity of carbon tetrachloride when given as a single dose before the toxin but not when given afterward.[113] Repeated dosing of DADS and DATS for 5−14 days before challenge with carbon tetrachloride gave protection, although DAS and DPTS had little or no effect.[127,173] The severity of the epithelial necrosis in the urinary bladder induced by cyclophosphamide in mice was decreased by DADS when given repetitively for 5 days before challenge. DAS was less effective.[174]

9. MECHANISMS OF PROTECTION AGAINST CARCINOGENS AND OTHER TOXIC CHEMICALS BY SULFIDES

Chemopreventative agents have been classified into two major categories (blockers and suppressors), depending upon the stage of the development of neoplasia at which they act.[175] Many carcinogens require activation before exerting their effects, and blocking agents may act by inhibiting the enzymes responsible for such activation. Blocking agents may also protect by detoxifying activated carcinogens. Activation of pro-carcinogens is a recognized feature of phase 1 enzymes, and these enzymes are also known to be involved in the activation of certain toxic chemicals. Phase 2 enzymes contribute to the detoxification of electrophilic activation products of pro-carcinogens and pro-toxicants. Blocking agents are effective when administered before or during exposure to the carcinogen. In contrast, suppressing agents are effective when given after initiation of the neoplastic process by carcinogens. They act by inducing differentiation or apoptosis or by inhibiting angiogenesis or oncogene activation.[175]

The chemopreventative activity of sulfides, particularly DAS, has frequently been attributed to inhibition of P450 2E1. This enzyme activates a number of carcinogens and toxicants, including AOM, DMH, vinyl carbamate, NDMA, NDEA, acetaminophen, and haloalkanes,[176,177] against which protection by sulfides has been demonstrated. The most convincing evidence for the involvement of P450 2E1 in protection would be demonstration of a protective effect of a single dose of the sulfide given shortly before a single dose of the toxicant. This has been demonstrated for DAS in protection against DMH,[155,156] vinyl carbamate,[164] acetaminophen,[171] and carbon tetrachloride,[113] indicating a major role for P450 2E1 under these

dx.doi.org/10.1021/tx200373u |*Chem. Res. Toxicol.* 2012, 25, 47–60

experimental conditions. This is consistent with the observations that diallyl sulfone, which is a more potent inhibitor of P450 2E1 than DAS,[178] was more effective in protecting against acetaminophen toxicity and that DADS, which is a weaker inhibitor of P450 2E1 than DAS, failed to protect against DMH. Furthermore, DAS gave no protection against the colonic preneoplastic changes induced by NMU and MNNG, which are both direct-acting carcinogens that need no activation. But the observation that DAS protected against the preneoplastic changes induced in the glandular stomach of rats given MNNG indicates that other mechanisms are operational in this instance.[157]

The role of P450 2E1 in protection against other toxic substances must be called into question. While P450 2E1 plays a major role in the activation of short-chain nitrosamines, the importance of this enzyme diminishes with increasing chain length, yet DAS protected against NMBA-induced esophageal cancer. Furthermore, enzymes of the P450 2B subfamily play an important role in the activation of NMBA and cyclophosphamide.[176,179,180] Since DAS increases the activity of enzymes in the P450 2B subfamily *in vivo*, it would be expected to increase the rate of activation of these substances and thereby increase their toxicity. The observed protection afforded by DAS must therefore involve other mechanisms, possibly involving metabolism of the carcinogen. DAS was shown to decrease the formation of acrolein, a toxic metabolite of cyclophosphamide, in mice, but the mechanism by which this occurs remains unknown.

Sulfides also increase the activity of enzymes of the P450 1 family of enzymes, yet they afford protection against the carcinogenicity of polycyclic hydrocarbons, NNK, PhIP, and IQ, which are activated by these enzymes.[176,177,181,182] In the case of benzo[*a*]pyrene, a correlation exists between the protective action of different sulfides against forestomach cancer and their ability to increase tissue activities of NQO1, and it has been suggested[183] that protection results from detoxification via this enzyme of the quinone metabolites of benzo[*a*]pyrene that are involved in its carcinogenic action.[184] Similarly, in the case of PhIP, protection has been attributed to the induction of phase 2 enzymes,[185] while DAS has been shown to inhibit the activation of NNK by an as yet unknown mechanism.[161,186]

Aflatoxin B_1 is activated by P450 2C11 in the rat,[177] but neither DAS nor DADS had any effect upon the activity of this enzyme, even though both of these substances decreased the incidence of aflatoxin-induced preneoplastic changes. Again, it has been suggested that such protection is largely due to increased activity of phase 2 enzymes, particularly aflatoxin B_1 aldehyde reductase.[152,187]

If phase 2 enzyme induction is of major importance, it would be expected that DATS and DADS would be more effective than DAS. This was the case for protection against the forestomach and mammary cancer induced by polycyclic hydrocarbons, the preneoplastic changes induced by AFB_1, the hepatotoxicity of carbon tetrachloride, and the damage to bladder epithelium induced by cyclophosphamide. But the effect of DADS against the colonic preneoplastic changes induced by AOM and against the carcinogenicity of DMH was no better than that of DAS, suggesting that phase 2 enzyme induction is less important in these situations.

Comparatively few experiments have been conducted on the suppression of carcinogenesis by sulfides, and experiments with DAS have given conflicting results. Jang et al.[188] reported that DAS fed to rats after initiation decreased the incidence of preneoplastic lesions induced by NDEA. The same authors showed that postinitiation administration of DAS decreased the incidence of hepatic hyperplastic nodules, pulmonary and thyroid adenomas, and papillary and nodular hyperplasia in the urinary bladder in a rat multiorgan carcinogenesis model.[189] In contrast, DAS and DPS were reported to increase the incidence of NDEA-induced preneoplastic changes in the livers of rats[169,190] and to increase the incidence of such changes after sequential administration of NDEA, NMU, *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine, and dihydroxy-di-*N*-propyl nitrosamine.[190] Results with disulfides are more consistent. Postinitiation administration of such substances was reported to decrease the incidence of hepatic preneoplastic changes induced by NDEA,[191] the incidence of aberrant crypt formation in the colon of rats dosed with AOM,[192] and the incidence of colonic and renal tumors after the administration of multiple carcinogens.[190]

Although the relative importance of the different mechanisms cannot be defined at the present time, there is evidence from animal experiments that sulfides do afford protection against carcinogens and other harmful chemicals. In general, allyl sulfides are the most effective, possibly reflecting their ability to both inhibit activation of certain carcinogens and to increase tissue levels of phase 2 enzymes. DADS and DATS were generally more effective than DAS, possibly because they have more pronounced effects on the latter enzymes. There is some evidence that disulfides act as suppressing agents *in vivo*, although there are reports that other sulfides, particularly monosulfides, act as promoters. Propyl derivatives, which have little effect on either phase 1 or phase 2 enzymes, are relatively ineffective. Little information is available on prop-1-enyl derivatives, but it is worth mentioning that in the first reported study on the anticancer effects of substances derived from *Allium* vegetables,[193] onion oil, which contains prop-1-enyl sulfides, was more effective than garlic oil, which contains mainly allyl sulfides. Further work on the onion-derived sulfides would be valuable.

10. CONCLUSIONS

Many sulfides to which humans and animals are exposed are toxic, and the evidence points to a role for ROS, which are formed by redox cycling of di- and polysulfides, in the toxic process. Polysulfides are more toxic than disulfides due to their metabolism to perthiols, which readily autoxidize to yield ROS, and the reactivity of perthiols has recently prompted the European Food Safety Authority to request more information on the toxicology of polysulfides used as food flavors.[194,195]

In animals, protection against toxic chemicals and carcinogens has been demonstrated, although there are some reports of an enhancing effect of monosulfides. Protection appears to involve, at least in part, inhibition of phase 1 enzymes that activate procarcinogens and the induction of phase 2 enzymes that detoxify electrophiles. The reason for enhancement by monosulfides is not known.

Epidemiological studies suggest that consumption of *Allium* vegetables may protect against cancer in humans, although more intervention trials, using the vegetables themselves or the major sulfides derived from them, are required. Such sulfides have been shown to protect against carcinogens in animals, but these data, while supporting a possible role for sulfides in cancer prevention, cannot be directly extrapolated to the human situation since very high dose-levels have generally been employed, which could not possibly be achieved by the consumption of *Allium* vegetables by humans. It is interesting, however, that significant increases in the

activity of phase 2 enzymes were observed in certain rat tissues at dose-levels of diallyl disulfide that could be achieved by the consumption of realistic amounts of garlic,[131] and it may be significant that in the epidemiological studies, the most consistent site showing a protective effect was the gastrointestinal tract, which, in animals, is the site at which sulfides were shown to exert their greatest effect on phase 2 enzyme activity. Studies on the effect of low levels of sulfides on chemically induced cancer in animals and on phase 2 enzyme activity in humans would be of great interest.

Di- and polysulfides have been shown to inhibit the growth of tumor xenografts in animals, by a mechanism possibly involving ROS, and it has been suggested that such compounds could be effective in cancer chemotherapy. If ROS are indeed involved, polysulfides would be the compounds of choice since they are the most active generators of such species. While allyl derivatives have been most extensively studied, methyl and propyl derivatives are equally effective producers of ROS. It must be remembered, however, that polysulfides have been shown to be potent hemolytic agents in animals, and while most humans appear to be relatively resistant to the hemolytic effects of sulfides, care should be taken if these compounds are used in individuals whose erythrocytes are deficient in glucose-6-phosphate dehydrogenase. Such individuals are highly susceptible to compounds that cause oxidative hemolysis,[196] and one such individual died after exposure to methanethiol, the reduction product of dimethyl disulfide.[197] Such considerations may also be of relevance to the use of romidepsin. Anemia has regularly been observed in phase 2 trials with this substance,[40,198,199] and the possibility of oxidative hemolysis should be considered.

It has also been suggested that epidithiodioxopiperazine derivatives could be valuable in cancer chemotherapy. While the anemia induced by simple sulfides is reversible, the liver damage induced by epidithiodioxopiperazines is not. Both gliotoxin and sporidesmin have been shown to cause severe toxic effects in animals, and it is possible that this is a common feature of this class of compound. Furthermore, very low doses of one such compound, sporidesmin, over a prolonged period leads to very severe toxic change.[200] Detailed toxicological studies will be needed before such compounds could be considered for use in humans.

AUTHOR INFORMATION

Corresponding Author

*Phone: + 64 7 838 5138. Fax: + 64 7 838 5012. E-mail: rex.munday@agresearch.co.nz.

ABBREVIATIONS

AFB_1, aflatoxin B_1; AOM, azoxymethane; DADS, diallyl disulfide; DAS, diallyl sulfide; DATS, diallyl trisulfide; DATTS, diallyl tetrasulfide; DMH, 1,2-dimethylhydrazine; DPDS, dipropyl disulfide; DPS, dipropyl sulfide; DPTS, dipropyl trisulfide; DPTTS, dipropyl tetrasulfide; EH, epoxide hydrolase; GST, glutathione *S*-transferase; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; MNU, *N*-methyl-*N*-nitrosourea; NDEA, *N*-nitrosodiethylamine; NMBA, *N*-nitrosomethylbenzylamine; NMDA, *N*-nitrosodimethylamine; NQO1, NAD(P)H: quinone acceptor oxidoreductase; PhIP, 2-amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine; ROS, reactive oxygen species; UGT, uridine diphosphate glucuronosyltransferase

REFERENCES

(1) Munday, R. (1989) Toxicity of thiols and disulphides: involvement of free-radical species. *Free Radical Biol. Med. 7*, 659–673.

(2) Schutte, L. (1974) Precursors of sulfur-containing flavor compounds. *Crit. Rev. Food Technol. 4*, 457–505.

(3) Burdock, G. A. (2010) *Fenaroli's Handbook of Flavor Ingredients*, 6th ed., CRC Press, Boca Raton, FL.

(4) Lawson, L. D. (1998) Garlic: A Review of Its Medicinal Effects and Indicated Active Compounds, in *Phytomedicines of Europe. Chemistry and Biological Activity* (Lawson, L. D., and Bauer, R., Eds.) ACS Symposium Series, 691, pp 176−209, American Chemical Society, Washington, D.C.

(5) Block, E. (2009) *Garlic and Other Alliums: The Lore and the Science*, RSC Publishing, Cambridge, England.

(6) Smith, R. H. (1978) *S*-Methylcysteine sulphoxide, the brassica anaemia factor (a valuable dietary factor for man?). *Vet. Sci. Comm. 2*, 47–61.

(7) Shirasu, M., Fujioka, K., Kakishima, S., Nagai, S., Tomizawa, Y., Tsukaya, H., Murata, J., Manome, Y., and Touhara, K. (2010) Chemical identity of a rotting animal-like odor emitted from the inflorescence of the Titan arum (*Amorphophallus titanum*). *Biosci., Biotechnol., Biochem. 74*, 2550–2554.

(8) Stensmyr, M. C., Urru, I., Collu, I., Celander, M., Hansson, B. S., and Angioy, A.-M. (2002) Rotting smell of dead-horse arum florets. *Nature 420*, 625–626.

(9) Jürgens, A., Dötterl, S., and Meve, U. (2006) The chemical nature of fetid floral odors in stapeliads (Apocynaceae-Asclepiadoideae-Ceropegieae). *New Phytologist 172*, 452–468.

(10) Wang, Y., Koreeda, M., Chatterji, T., and Gates, K. S. (1998) Total synthesis and DNA-cleaving properties of thiarubrine C. *J. Org. Chem. 63*, 8644–8645.

(11) Chen, C.-C., and Ho, C.-T. (1986) Identification of sulfurous compounds of Shiitake mushroom (*Lentinus edodes* Sing.). *J. Agric. Food Chem. 34*, 830–833.

(12) Nagarajan, R. (1984) Gliotoxin and Epipolythiodioxopiperazines, in *Mycotoxins: Production, Isolation, Separation and Purification* (Betina, V., Ed.) pp 351−385, Elsevier, Amsterdam.

(13) Ueda, H., Nakajima, H., Hori, Y., Fujita, T., Nishimura, M., Goto, T., and Okuhara, M. (1994) FR901228, a novel antitumor bicyclic depsipeptide produced by *Chromobacterium violaceum* No. 968. 1. Taxonomy, fermentation, isolation, physico-chemical and biological properties, and antitumor activity. *J. Antibiot. 47*, 301–310.

(14) Hara, M., Asano, K., Kawamoto, I., Takiguchi, T., Katsumata, S., Takahashi, K.-I., and Nakano, H. (1989) Leinamycin, a new antitumor antibiotic from *Streptomyces*; producing organism, fermentation and isolation. *J. Antibiot. 42*, 1768–1774.

(15) Davidson, B. S., Molinski, T. F., Barrows, L. R., and Ireland, C. M. (1991) Varacin: a novel benzopentathiepin from *Lissoclinum vareau* that is cytotoxic toward a human colon tumor. *J. Am. Chem. Soc. 113*, 4709–4710.

(16) Searle, P. A., and Molinski, T. F. (1994) Five new alkaloids from the tropical ascidian, *Lissoclinum* sp. Lissoclinotoxin A is chiral. *J. Org. Chem. 59*, 6600–6605.

(17) Anderson, K. K., and Bernstein, D. T. (1975) Some chemical constituents of the scent of the striped skunk (*Mephitis mephitis*). *J. Chem. Ecol. 1*, 493–499.

(18) Dugravot, S., Mondy, N., Mandon, N., and Thibout, E. (2005) Increased sulfur precursors and volatiles production by the leek *Allium porrum* in response to specialist insect attack. *J. Chem. Ecol. 31*, 1299–1314.

(19) Akdeniz, N., Koziel, J. A., Ahn, H.-K., Glanville, T. D., Crawford, B. P., and Raman, D. J. (2009) Air sampling and analysis method for volatile organic compounds (VOCs) related to field-scale mortality composting operations. *J. Agric. Food Chem. 57*, 5658–5664.

(20) Kwon-Chung, K. J., and Sugui, J. A. (2009) What do we know about the role of gliotoxin in the pathobiology of *Aspergillus fumigatus*? *Med. Mycol. 47* (Suppl. 1), S97–S103.

(21) di Menna, M. E., Smith, B. L., and Miles, C. O. (2009) A history of facial eczema (pithomycotoxicosis) research. *N. Z. J. Agric. Res. 52*, 345–376.

(22) Teyssier, C., and Siess, M.-H. (2000) Metabolism of dipropyl disulfide by rat liver phase I and phase II enzymes and by isolated perfused rat liver. *Drug Metab. Dispos. 28*, 648–654.
(23) Munday, R. (1994) Bioactivation of Thiols by One-Electron Oxidation, in *Conjugation-Dependent Carcinogenicity and Toxicity of Foreign Compounds* (Anders, M. W., and Dekant, W., Eds.) pp 237–270, Academic Press, San Diego, CA.
(24) Danehy, J. P., and Parameswaran, K. N. (1968) Acidic dissociation constants of thiols. *J. Chem. Eng. Data 13*, 386–389.
(25) Munday, R. (1985) Toxicity of aromatic disulphides. I. Generation of superoxide radical and hydrogen peroxide by aromatic disulphides *in vitro*. *J. Appl. Toxicol. 5*, 402–408.
(26) Everett, S. A., and Wardman, P. (1995) Perthiols as antioxidants: radical scavenging and prooxidative mechanisms. *Methods Enzymol. 251*, 55–69.
(27) Kawamura, S., Abe, Y., and Tsurugi, J. (1969) Aralkyl hydrodisulfides. X. Reactions with iron salts. *J. Org. Chem. 34*, 3633–3635.
(28) Munday, R., Munday, J. S., and Munday, C. M. (2003) Comparative effects of mono-, di-, tri-, and tetrasulfides derived from plants of the *Allium* family: redox cycling in vitro and hemolytic activity and phase 2 enzyme induction in vivo. *Free Radical Biol. Med. 34*, 1200–1211.
(29) Ronaldson, J. W. (1978) Fungal Metabolite Chemistry: Sporidesmin, Crepidotine, Ph.D. dissertation, Waikato University, Hamilton, New Zealand.
(30) Munday, R. (1982) Studies on the mechanism of toxicity of the mycotoxin, sporidesmin. I. Generation of superoxide radical by sporidesmin. *Chem.-Biol. Interact. 41*, 361–374.
(31) Eichner, R. D., Waring, P., Geue, A. M., Braithwaite, A. W., and Müllbacher, A. (1988) Gliotoxin causes oxidative damage to plasmid and cellular DNA. *J. Biol. Chem. 263*, 3772–3777.
(32) Munday, R. (1987) Studies on the mechanism of toxicity of the mycotoxin sporidesmin. V. Generation of hydroxyl radical by sporidesmin. *J. Appl. Toxicol. 7*, 17–22.
(33) Munday, R. (1984) Studies on the mechanism of toxicity of the mycotoxin sporidesmin. 3. Inhibition by metals of the generation of superoxide radical by sporidesmin. *J. Appl. Toxicol. 4*, 182–186.
(34) Woodcock, J. C., Henderson, W., and Miles, C. O. (2001) Metal complexes of the mycotoxins sporidesmin A and gliotoxin, investigated by electrospray ionisation mass spectrometry. *J. Inorg. Biochem. 85*, 187–199.
(35) Chatterji, T., and Gates, K. S. (1998) DNA cleavage by 7-methylbenzopentathiepin: a simple analog of the antitumor antibiotic varacin. *Bioorg. Med. Chem. Lett. 8*, 535–538.
(36) Chatterji, T., and Gates, K. S. (2003) Reaction of thiols with 7-methylbenzopentathiepin. *Bioorg. Med. Chem. Lett. 13*, 1349–1352.
(37) Lee, A. H. F., Chan, A. S. C., and Li, T. (2002) Acid-accelerated DNA-cleaving activities of antitumor antibiotic varacin. *Chem. Commun.* 2112–2113.
(38) Sivaramakrishnan, S., and Gates, K. S. (2008) Possible chemical mechanisms underlying the antitumor activity of *S*-deoxyleinamycin. *Bioorg. Med. Chem. Lett. 18*, 3076–3080.
(39) Gates, K. S. (2000) Mechanisms of DNA damage by leinamycin. *Chem. Res. Toxicol. 13*, 953–956.
(40) Mizutani, H., Hiraku, Y., Tada-Oikawa, S., Murata, M., Ikemura, K., Iwamoto, T., Kagawa, Y., Okuda, M., and Kawanishi, S. (2010) Romidepsin (FK228), a potent histone deacetylase inhibitor, induces apoptosis through the generation of hydrogen peroxide. *Cancer Sci. 101*, 2214–2219.
(41) Munday, R., Manns, E., and Fowke, E. A. (1990) Steric effects on the haemolytic activity of aromatic disulphides in rats. *Food Chem. Toxicol. 28*, 561–566.
(42) Munday, R. (1985) Toxicity of aromatic disulphides. II. Intraerythrocytic hydrogen peroxide formation and oxidative damage by aromatic disulphides. *J. Appl. Toxicol. 5*, 409–413.
(43) Wang, H.-C., Yang, J.-H., Hsieh, S.-C., and Sheen, L.-Y. (2010) Allyl sulfides inhibit cell growth of skin cancer cells through induction of DNA damage mediated G_2/M arrest and apoptosis. *J. Agric. Food Chem. 58*, 7096–7103.
(44) Wu, X.-J., Hu, Y., Lamy, E., and Mersch-Sundermann, V. (2009) Apoptosis induction in human lung adenocarcinoma cells by oil-soluble allyl sulfides: triggers, pathways, and modulators. *Env. Mol. Mutagen. 50*, 266–275.
(45) Yi, L., Ji, X.-X., Lin, M., Tan, H., Tang, Y., Wen, L., Ma, Y.-H., and Su, Q. (2010) Diallyl disulfide induces apoptosis in human leukemia HL-60 cells through activation of JNK mediated by reactive oxygen. *Pharmazie 65*, 693–698.
(46) Viswesh, V., Gates, K., and Sun, D. (2010) Characterization of DNA damage induced by a natural product antitumor antibiotic leinamycin in human cancer cells. *Chem. Res. Toxicol. 23*, 99–107.
(47) Munday, R. (1984) Studies on the mechanism of toxicity of the mycotoxin sporidesmin. 2. Evidence for intracellular generation of superoxide radical from sporidesmin. *J. Appl. Toxicol. 4*, 176–181.
(48) Bible, K. C., Isham, C. R., Xu, R., and Tibodeau, J. D. (2009) Methods and compositions for treating cancer. US Patent Application 2009/0264421.
(49) Antosiewicz, J., Ziolkowski, W., Kar, S., Powolny, A. A., and Singh, S. V. (2008) Role of reactive oxygen intermediates in cellular responses to dietary cancer chemopreventive agents. *Planta Med. 74*, 1570–1579.
(50) Scherer, C., Jacob, C., Dicato, M., and Diederich, M. (2009) Potential role of organic sulfur compounds from *Allium* species in cancer prevention and therapy. *Phytochem. Rev. 8*, 349–368.
(51) Wu, X., Kassie, F., and Mersch-Sundermann, V. (2005) Induction of apoptosis in tumor cells by naturally occurring sulfur-containing compounds. *Mutat. Res. 589*, 81–102.
(52) Cerella, C., Dicato, M., Jacob, C., and Diederich, M. (2011) Chemical properties and mechanisms determining the anti-cancer action of garlic-derived organic sulfur compounds. *Anti-Cancer Agents Med. Chem. 11*, 267–271.
(53) Zhang, G., Wu, H., Zhu, B., Shimoishi, Y., Nakamura, Y., and Murata, Y. (2008) Effect of dimethyl sulfides on the induction of apoptosis in human leukemia Jurkat cells and HL-60 cells. *Biosci., Biotechnol., Biochem. 72*, 2966–2972.
(54) Busch, C., Jacob, C., Anwar, A., Burkholz, T., Ba, L. A., Cerella, C., Diederich, M., Brandt, W., Wessjohann, L., and Montenarh, M. (2010) Diallylpolysulfides induce growth arrest and apoptosis. *Int. J. Oncol. 36*, 743–749.
(55) Hileman, E. O., Liu, J., Albitar, M., Keating, M. J., and Huang, P. (2004) Intrinsic oxidative stress in cancer cells: a biochemical basis for therapeutic selectivity. *Cancer Chemother. Pharmacol. 53*, 209–219.
(56) Hail, N., Cortes, M., Drake, E. N., and Spallholz, J. E. (2008) Cancer chemoprevention: a *radical* perspective. *Free Radical Biol. Med. 45*, 97–110.
(57) Hara, M., Saitoh, Y., and Nakano, H. (1990) DNA strand scission by the novel antitumor antibiotic leinamycin. *Biochemistry 29*, 5676–5681.
(58) Jain, S., and Zain, J. (2011) Romidepsin in the treatment of cutaneous T-cell lymphoma. *J. Blood Med. 2*, 37–47.
(59) Gardiner, D. M., Waring, P., and Howlett, B. J. (2005) The epipolythiodioxopiperazine (ETP) class of fungal toxins: distribution, mode of action, functions and biosynthesis. *Microbiology 151*, 1021–1032.
(60) Isham, C. R., Tibodeau, J. D., Jin, W., Xu, R., Timm, M. M., and Bible, K. C. (2007) Chaetocin: a promising new antimyeloma agent with in vitro and in vivo activity mediated via imposition of oxidative stress. *Blood 109*, 2579–2588.
(61) Choi, E. J., Park, J.-S., Kim, Y.-J., Jung, J.-H., Lee, J. K., Kwon, H. C., and Yang, H. O. (2010) Apoptosis-inducing effect of diketopiperazine disulfides produced by *Aspergillus* sp. KMD 901 isolated from marine sediment on HCT116 colon cancer cell lines. *J. Appl. Microbiol. 110*, 304–313.
(62) Wong, W. W.-L., Macdonald, S., Langler, R. F., and Penn, L. Z. (2000) Novel synthetic organosulfur compounds induce apoptosis of human leukemic cells. *Anticancer Res. 20*, 1367–1374.
(63) Verma, R. P., Kapur, S., Barberena, O., Shusterman, A., Hansch, C. H., and Selassie, C. D. (2003) Synthesis, cytotoxicity, and QSAR analysis of X-thiophenols in rapidly dividing cells. *Chem. Res. Toxicol. 16*, 276–284.
(64) Hiramitsu, T., and Sakamoto, K.-I. (1991) (Trans-1-propenyl) disulfide derivative and process for preparing the same. US Patent 5,011,975.

(65) Xiao, D., Lew, K. L., Kim, Y.-A., Zeng, Y., Hahm, E.-R., Dhir, R., and Singh, S. V. (2006) Diallyl trisulfide suppresses growth of PC-3 human prostate cancer xenograft *in vivo* in association with Bax and Bak induction. *Clin. Cancer Res. 12*, 6836–6843.

(66) Shankar, S., Chen, Q., Ganapathy, S., Singh, K. P., and Srivastava, R. K. (2008) Diallyl trisulfide increases the effectiveness of TRAIL and inhibits prostate cancer growth in an orthotopic model: molecular mechanisms. *Mol. Cancer Ther. 7*, 2328–2338.

(67) Zhang, Z.-M., Yang, X.-Y., Deng, S.-H., Xu, W., and Gao, H.-Q. (2007) Anti-tumor effects of polybutylcyanoacrylate nanoparticles of diallyl trisulfide on orthotopic transplantation tumor model of hepatocellular carcinoma in BALB/c nude mice. *Chin. Med. J. 120*, 1336–1342.

(68) Zhao, J., Huang, W.-G., He, J., Tan, H., Liao, Q.-J., and Su, Q. (2006) Diallyl disulfide suppresses growth of HL-60 cell through increasing histone acetylation and $p21^{WAF1}$ expression *in vivo* and *in vitro*. *Acta Pharmacol. Sin. 27*, 1459–1466.

(69) Xu, W., Xi, B., Wu, J., An, H., Zhu, J., Abassi, Y., Feinstein, S. C., Gaylord, M., Geng, B., Yan, H., Fan, W., Sui, M., Wang, X., and Xu, X. (2009) Natural product derivative bis(4-fluorobenzyl)trisulfide inhibits tumor growth by modification of β-tubulin at Cys 12 and suppression of microtubule dynamics. *Mol. Cancer Ther. 8*, 3318–3330.

(70) Pan, X. Q., and Harday, J. (2007) Electromicroscopic observations on gliotoxin-induced apoptosis of cancer cells in culture and human cancer xenografts in transplanted SCID mice. *In Vivo 21*, 259–266.

(71) Vigushin, D. M., Mirsaidi, N., Brooke, G., Sun, C., Pace, P., Inman, L., Moody, C. J., and Coombes, R. C. (2004) Gliotoxin is a dual inhibitor of farnesyltransferase and geranylgeranyltransferase I with antitumor activity against breast cancer in vivo. *Med. Oncol. 21*, 21–30.

(72) Lee, Y.-M., Lim, J.-H., Yoon, H., Chun, Y.-S., and Park, J.-W. (2011) Antihepatoma activity of chaetocin due to deregulated splicing of hypoxia-inducible factor 1α pre-mRNA in mice and *in vitro*. *Hepatology 53*, 171–180.

(73) Kung, A. L., Zabludoff, S. D., France, D. S., Freedman, S. J., Tanner, E. A., Vieira, A., Cornell-Kennon, S., Lee, J., Wang, B., Wang, J., Memmert, K., Naegeli, H.-U., Petersen, F., Eck, M. J., Bair, K. W., Wood, A. W., and Livingston, D. M. (2004) Small molecule blockade of transcriptional coactivation of the hypoxia-inducible factor pathway. *Cancer Cell 6*, 33–43.

(74) Powolny, A. A., and Singh, S. V. (2008) Multitargeted prevention and therapy of cancer by diallyl trisulfide and related *Allium* vegetable-derived organosulfur compounds. *Cancer Lett. 269*, 305–314.

(75) Rifkind, R. A., and Danon, D. (1965) Heinz body anemia: an ultrastructural study. I. Heinz body formation. *Blood 25*, 885–896.

(76) Hasegawa, S., Rodgers, G. P., Shio, H., Schechter, A. N., and Uyesaka, N. (1993) Impaired deformability of Heinz body-forming red cells. *Biorheology 30*, 275–286.

(77) Rifkind, R. A. (1965) Heinz body anemia: an ultrastructural study. II. Red cell sequestration and destruction. *Blood 26*, 433–448.

(78) Jenkins, F. P., Robinson, J. A., Gellatly, J. B. M., and Salmond, G. W. A. (1972) The no-effect dose of aniline in human subjects and a comparison of aniline toxicity in man and the rat. *Food Cosmet. Toxicol. 10*, 671–679.

(79) Roque, M., D'Anna, C., Gatti, C., and Veuthey, T. (2008) Hematological and morphological analysis of the erythropoietic regenerative response in phenylhydrazine-induced hemolytic anemia in mice. *Scand. J. Lab. Anim. Sci. 35*, 181–190.

(80) Borelli, V., Lucioli, J., Furlan, F. H., Hoepers, P. G., Roveda, J. F., Traverso, S. D., and Gava, A. (2009) Fatal onion (*Allium cepa*) toxicosis in water buffalo (*Bubalus bubalis*). *J. Vet. Diagn. Invest. 21*, 402–405.

(81) Salgado, B. S., Monteiro, L. N., and Rocha, N. S. (2011) *Allium* species poisoning in dogs and cats. *J. Venomous Anim. Toxins Incl. Trop. Dis. 17*, 4–11.

(82) Aslani, M. R., Mohri, M., and Movassaghi, A. R. (2005) Heinz body anaemia associated with onion (*Allium cepa*) toxicosis in a flock of sheep. *Comp. Clin. Pathol. 14*, 118–120.

(83) Pearson, W., Boermans, H. J., Bettger, W. J., McBride, B. W., and Lindinger, M. I. (2005) Association of maximum voluntary dietary intake of freeze-dried garlic with Heinz body anemia in horses. *Am. J. Vet. Res. 66*, 457–465.

(84) Ostrowska, E., Gabler, N. K., Sterling, S. J., Tatham, B. G., Jones, R. B., Eagling, D. R., Jois, M., and Dunshea, F. R. (2004) Consumption of brown onions (*Allium cepa* var. *cavalier* and var. *destiny*) moderately modulates blood lipids, haematological and haemostatic variables in healthy pigs. *Br. J. Nutr. 91*, 211–218.

(85) Gebauer, H., and Ploetz, W. (1954) Zweibelanämie, Vitamin B_{12} und Folsäure. *Pharmazie 9*, 475–480.

(86) Crespo, R., and Chin, R. P. (2004) Effect of feeding green onions (*Allium ascalonicum*) to white Chinese geese (*Threskiornis spinicollis*). *J. Vet. Diagn. Invest. 16*, 321–325.

(87) Baldissera Nordio, C. (1952) Ricerche sul comportamento ematologico dei polli trattati con *Allium cepa*. *Boll. Soc. Ital. Biol. Sper. 28*, 1008–1009.

(88) Nakagawa, S., Masamoto, K., Sumiyoshi, H., Kunihiro, K., and Fuwa, T. (1980) Effect of raw and extracted-aged garlic juice on growth of young rats and their organs after peroral administration. *J. Toxicol. Sci. 5*, 91–112.

(89) Akgül, B., Lin, K.-W., Yang, H.-M. O., Chen, Y.-H., Lu, T.-H., Chen, C.-H., Kikuchi, T., Chen, Y.-T., and Tu, C.-P. D. (2010) Garlic accelerates red blood cell turnover and splenic erythropoietic gene expression in mice: evidence for erythropoietin-independent erythropoiesis. *PLoS One 5*, e15358.

(90) Rae, H. A. (1999) Onion toxicosis in a herd of beef cows. *Can. Vet. J. 40*, 55–57.

(91) Kaplan, A. J. (1995) Onion powder in baby food may induce anemia in cats. *J. Am. Vet. Med. Ass. 207*, 1405.

(92) Robertson, J. E., Christopher, M. M., and Rogers, Q. R. (1998) Heinz body formation in cats fed baby food containing onion powder. *J. Am. Vet. Med. Ass. 212*, 1260–1266.

(93) Šućur, M. (1980) Effect of garlic on serum lipids and lipoproteins in patients suffering from hyperlipoproteinemia. *Diabetol. Croatica 9*, 323–338.

(94) Gadkari, J. V., and Joshi, V. D. (1991) Effect of ingestion of raw garlic on serum cholesterol level, clotting time and fibrinolytic activity in normal subjects. *J. Postgrad. Med. 37*, 128–131.

(95) Gruhzit, O. M. (1931) Anemia in dogs produced by feeding disulphide compounds. *Am. J. Med. Sci. 181*, 815–820.

(96) Kobayashi, K. (1981) Onion poisoning in the cat. *Feline Pract. 11*, 22–27.

(97) Munday, R., and Manns, E. (1994) Comparative toxicity of prop(en)yl disulfides derived from Alliaceae: possible involvement of 1-propenyl disulphides in onion-induced hemolytic anemia. *J. Agric. Food Chem. 42*, 959–962.

(98) Zaks, K. L., Tan, E. O., and Thrall, M. A. (2005) Heinz body anemia in a dog that had been sprayed with skunk musk. *J. Am. Vet. Med. Assoc. 226*, 1516–1518.

(99) Munday, R., and Manns, E. (1985) Toxicity of aromatic disulphides. III. *In vivo* haemolytic activity of aromatic disulphides. *J. Appl. Toxicol. 5*, 414–417.

(100) Munday, R. (1995) *In vivo* toxicity of thiols: relationship to rate of one-electron oxidation by oxyhemoglobin. *Methods Enzymol. 251*, 117–120.

(101) Munday, R., Munday, C. M., and Munday, J. S. (2005) Hemolytic anemia and induction of phase II detoxification enzymes by diprop-1-enyl sulfide in rats: dose-response study. *J. Agric. Food Chem. 53*, 9695–9700.

(102) Munday, R., and Munday, J. S. (2003) Comparative haemolytic activity of bis(phenylmethyl) disulphide, bis(phenylethyl) disulphide and bis(phenylpropyl) disulphide in rats. *Food Chem. Toxicol. 41*, 1609–1615.

(103) Smith, B. L., and Towers, N. R. (2002) Mycotoxicoses of grazing animals in New Zealand. *N. Z. Vet. J. 50* (Suppl.), 28–34.

(104) Mortimer, P. H., and Stanbridge, T. A. (1968) The excretion of sporidesmin given by mouth to sheep. *J. Comp. Pathol. 78*, 505–512.

(105) Mortimer, P. H. (1963) The experimental intoxication of sheep with sporidesmin, a metabolic product of *Pithomyces chartarum*. IV.

Histological and histochemical examinations of orally-dosed sheep. *Res. Vet. Sci. 4*, 166–185.

(106) Frame, R., and Carlton, W. W. (1988) Acute toxicity of gliotoxin in hamsters. *Toxicol. Lett. 40*, 269–273.

(107) Parola, M., Cheeseman, K. H., Biocca, M. E., Dianzani, M. U., and Slater, T. F. (1990) Menadione and cumene hydroperoxide induced cytotoxicity in biliary epithelial cells isolated from rat liver. *Biochem. Pharmacol. 39*, 1727–1734.

(108) Munday, R., Thompson, A. M., Smith, B. L., Towers, N. R., O'Donnell, K., McDonald, R. M., and Stirnemann, M. (2001) A zinc-containing intraruminal device for prevention of the sporidesmin-induced cholangiopathy of facial eczema in calves. *N. Z. Vet. J. 49*, 29–33.

(109) Munday, R., and Manns, E. (1989) Protection by iron salts against sporidesmin intoxication in sheep. *N. Z. Vet. J. 37*, 65–68.

(110) Munday, R., Manns, E., and Mortimer, P. H. (1983) Effect of antioxidants on the toxicity of the facial eczema toxin, sporidesmin, in sheep. *Proc. N. Z. Soc. Anim. Prod. 43*, 209–212.

(111) Zeng, T., and Xie, K.-Q. (2010) The differential modulation on cytochrome P450 enzymes by garlic components. *Food Rev. Int. 26*, 353–363.

(112) Forkert, P.-G., Premdas, P. D., and Bowers, R. J. (2000) Epoxide formation from diallyl sulfone is associated with CYP2E1 inactivation in murine and human lungs. *Am. J. Respir. Cell Mol. Biol. 23*, 687–695.

(113) Brady, J. F., Wang, M.-H., Hong, J.-Y., Xiao, F., Li, Y., Yoo, J.-S. H., Ning, S. M., Lee, M.-J., Fukuto, J. M., Gapac, J. M., and Yang, C. S. (1991) Modulation of rat hepatic microsomal monooxygenase enzymes and cytotoxicity by diallyl sulfide. *Toxicol. Appl. Pharmacol. 108*, 342–354.

(114) Chen, L., Lee, M., Hong, J.-Y., Huang, W., Wang, E., and Yang, C. S. (1994) Relationship between cytochrome P450 2E1 and acetone catabolism in rats as studied with diallyl sulfide as an inhibitor. *Biochem. Pharmacol. 48*, 2199–2205.

(115) Haber, D., Siess, M.-H., Canivenc-Lavier, M.-C., Le Bon, A.-M., and Suschetet, M. (1995) Differential effects of dietary diallyl sulfide and diallyl disulfide on rat intestinal and hepatic drug-metabolizing enzymes. *J. Toxicol. Env. Health 44*, 423–434.

(116) Křížková, J. J., Burdová, K., Hudeček, J., Stiborová, M., and Hodek, P. (2008) Induction of cytochromes P450 in small intestine by chemopreventive compounds. *Neuroendocrinol. Lett. 29*, 717–721.

(117) Pan, J., Hong, J.-Y., Ma, B.-L., Ning, S. M., Paranawithana, S. R., and Yang, C. S. (1993) Transcriptional activation of cytochrome P450 2B1/2 genes in rat liver by diallyl sulfide, a compound derived from garlic. *Arch. Biochem. Biophys. 302*, 337–342.

(118) Lii, C.-K., Tsai, C.-W., and Wu, C.-C. (2006) Garlic allyl sulfides display differential modulation of rat cytochrome P450 2B1 and the placental form glutathione *S*-transferase in various organs. *J. Agric. Food Chem. 54*, 5191–5196.

(119) Srivastava, S. K., Hu, X., Xia, H., Zaren, H. A., Chatterjee, M. L., Agarwal, R., and Singh, S. V. (1997) Mechanism of differential efficacy of garlic organosulfides in preventing benzo(*a*)pyrene-induced cancer in mice. *Cancer Lett. 118*, 61–67.

(120) Gurley, B. J., Gardner, S. F., Hubbard, M. A., Williams, D. K., Gentry, W. B., Cui, Y., and Ang, C. Y. W. (2005) Clinical assessment of effects of botanical supplementation on cytochrome P450 phenotypes in the elderly. *Drugs Aging 22*, 525–539.

(121) Wilkinson, G. R. (1997) The effects of diet, aging and disease-states on presystemic elimination and oral drug bioavailability in humans. *Adv. Drug Delivery Rev. 27*, 129–159.

(122) Talalay, P., Dinkova-Kostova, A. T., and Holtzclaw, W. D. (2003) Importance of phase 2 gene regulation in protection against electrophile and reactive oxygen toxicity and carcinogenesis. *Adv. Enzyme Reg. 43*, 121–134.

(123) Talalay, P. (2000) Chemoprotection against cancer by induction of Phase 2 enzymes. *BioFactors 12*, 5–11.

(124) Kensler, T. W., and Wakabayashi, N. (2010) Nrf2: friend or foe for chemoprevention? *Carcinogenesis 31*, 90–99.

(125) Kwak, M.-K., and Kensler, T. W. (2010) Targeting NRF2 signaling for cancer chemoprevention. *Toxicol. Appl. Pharmacol. 244*, 66–76.

(126) Munday, R., and Munday, C. M. (2004) Induction of Phase II enzymes by aliphatic sulfides derived from garlic and onions: an overview. *Methods Enzymol. 382*, 449–456.

(127) Hosono-Fukao, T., Hosono, T., Seki, T., and Ariga, T. (2009) Diallyl trisulfide protects rats from carbon tetrachloride-induced liver injury. *J. Nutr. 139*, 2252–2256.

(128) Haber, D., Siess, M.-H., de Waziers, I., Beaune, P., and Suschetet, M. (1994) Modification of hepatic drug-metabolizing enzymes in rat fed naturally occurring allyl sulphides. *Xenobiotica 24*, 169–182.

(129) Sheen, L.-Y., Chen, H.-W., Kung, Y.-L., Liu, C.-T., and Lii, C.-K. (1999) Effects of garlic oil and its organosulfur compounds on the activities of hepatic drug-metabolizing and antioxidant enzymes in rats fed high- and low-fat diets. *Nutr. Cancer 35*, 160–166.

(130) Reddy, B. S., Rao, C. V., Rivenson, A., and Kelloff, G. (1993) Chemoprevention of colon carcinogenesis by organosulfur compounds. *Cancer Res. 53*, 3493–3498.

(131) Munday, R., and Munday, C. M. (1999) Low doses of diallyl disulfide, a compound derived from garlic, increase tissue activities of quinone reductase and glutathione transferase in the gastrointestinal tract of the rat. *Nutr. Cancer 34*, 42–48.

(132) Slocum, S. L., and Kensler, T. W. (2011) Nrf2: control of sensitivity to carcinogens. *Arch. Toxicol. 85*, 273–284.

(133) Sekhar, K. R., Rachakonda, G., and Freeman, M. L. (2010) Cysteine-based regulation of the CUL3 adaptor protein Keap1. *Toxicol. Appl. Pharmacol. 244*, 21–26.

(134) Kaspar, J. W., Niture, S. K., and Jaiswal, A. K. (2009) Nrf2: INrf2 (Keap1) signaling in oxidative stress. *Free Radical Biol. Med. 47*, 1304–1309.

(135) Fisher, C. D., Augustine, L. M., Maher, J. M., Nelson, D. M., Slitt, A. L., Klaassen, C. D., Lehman-McKeeman, L. D., and Cherrington, N. J. (2007) Induction of drug-metabolizing enzymes by garlic and allyl sulfide compounds via activation of constitutive androstane receptor and nuclear factor E2-related factor 2. *Drug Metab. Dispos. 35*, 995–1000.

(136) Chen, C., Pung, D., Leong, V., Hebbar, V., Shen, G., Nair, S., Li, W., and Kong, A.-N. T. (2004) Induction of detoxifying enzymes by garlic organosulfur compounds through transcription factor NRF2: effect of chemical structure and stress signals. *Free Radical Biol. Med. 37*, 1578–1590.

(137) You, W.-C., Blot, W. J., Chang, Y.-S., Ershow, A., Yang, Z. T., An, Q., Henderson, B. E., Fraumeni, J. F., and Wang, T.-G. (1989) Allium vegetables and reduced risk of stomach cancer. *J. Natl Cancer Inst. 81*, 162–164.

(138) Bianchini, F., and Vaino, H. (2001) *Allium* vegetables and organosulfur compounds: do they help prevent cancer? *Env. Health Perspect. 109*, 893–902.

(139) World Cancer Research Fund/American Institute for Cancer Research (2007) *Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective*, AICR, WA, Washington, D.C.

(140) Kim, J. Y., and Kwon, O. (2009) Garlic intake and cancer risk: an analysis using the Food and Drug Administration's evidence-based review system for the scientific evaluation of health claims. *Am. J. Clin. Nutr. 89*, 257–264.

(141) Zhou, Y., Zhuang, W., Hu, W., Liu, G.-J., Wu, T.-X., and Wu, X.-T. (2011) Consumption of large amounts of *Allium* vegetables reduces risk for gastric cancer in a meta-analysis. *Gastroenterology 141*, 80–89.

(142) Li, H., Li, H.-Q., Wang, Y., Xu, H.-X., Fan, W.-T., Wang, M.-L., Sun, P.-H., and Xie, X.-Y. (2004) An intervention study to prevent gastric cancer by micro-selenium and large dose of allitridum. *Chin. Med. J. 117*, 1155–1160.

(143) You, W.-C., Brown, L. M., Zhang, L., Li, J.-Y., Jin, M.-L., Chang, Y.-S., Ma, J.-L., Pan, K.-F., Liu, W.-D., Hu, Y., Crystal-Mansour, S., Pee, D., Blot, W. J., Fraumeni, J. F., Xu, G.-W., and Gail, M. H. (2006) Randomized double-blind factorial trial of three treatments to reduce the prevalence of precancerous gastric lesions. *J. Natl. Cancer Inst. 98*, 974–983.

(144) Lau, B. H. S., Tadi, P. P., and Tosk, J. M. (1990) Allium sativum (garlic) and cancer prevention. *Nutr. Res. (N.Y.) 10*, 937–948.

(145) Imai, J., Ide, N., Nagae, S., Moriguchi, T., Matsuura, H., and Itakura, Y. (1994) Antioxidant and radical scavenging effects of aged garlic extract and its constituents. *Planta Med. 60*, 417–420.

(146) Lawson, L. D. (1996) The Composition and Chemistry of Garlic Cloves and Processed Garlic, in *Garlic: The Science and Therapeutic Application of Allium sativum L. and Related Species*, 2nd ed. (Koch, H. P., and Lawson, L. D., Eds.) pp 37−107, Williams & Wilkins, Baltimore, MD.

(147) Singh, A., and Shukla, Y. (1998) Antitumour activity of diallyl sulfide on polycyclic aromatic hydrocarbon-induced mouse skin carcinogenesis. *Cancer Lett. 131*, 209–214.

(148) Belman, S., Solomon, J., Segal, A., Block, E., and Barany, G. (1989) Inhibition of soybean lipoxygenase and mouse skin tumor promotion by onion and garlic components. *J. Biochem. Toxicol. 4*, 151–160.

(149) Shrotriya, S., Kundu, J. K., Na, H.-K., and Surh, Y.-J. (2010) Diallyl trisulfide inhibits phorbol ester-induced tumor promotion, activation of AP-1, and expression of COX-2 in mouse skin by blocking JNK and Akt signaling. *Cancer Res. 70*, 1932–1940.

(150) Ip, C., Lisk, D. J., and Stoewsand, G. S. (1992) Mammary cancer prevention by regular garlic and selenium-enriched garlic. *Nutr. Cancer 17*, 279–286.

(151) Sparnins, V. L., Barany, G., and Wattenberg, L. W. (1988) Effects of organosulfur compounds from garlic and onions on benzo[*a*]pyrene-induced neoplasia and glutathione *S*-transferase activity in the mouse. *Carcinogenesis 9*, 131–134.

(152) Haber-Mignard, D., Suschetet, M., Bergès, R., Astorg, P., and Siess, M.-H. (1996) Inhibition of aflatoxin B_1- and *N*-nitrosodiethylamine-induced liver preneoplastic foci in rats fed naturally occurring allyl sulfides. *Nutr. Cancer 25*, 61–70.

(153) Le Bon, A.-M., Roy, C., Dupont, C., and Suschetet, M. (1997) In vivo antigenotoxic effects of dietary allyl sulfides in the rat. *Cancer Lett. 114*, 131–134.

(154) Sengupta, A., Ghosh, S., Das, R. K., Bhattacharjee, S., and Bhattacharya, S. (2006) Chemopreventive potential of diallylsulfide, lycopene and theaflavin during chemically induced colon carcinogenesis in rat colon through modulation of cyclooxygenase-2 and inducible nitric oxide synthase pathways. *Eur. J. Cancer Prev. 15*, 301–305.

(155) Sumiyoshi, H., and Wargovich, M. J. (1990) Chemoprevention of 1,2-dimethylhydrazine-induced colon cancer in mice by naturally occurring organosulfur compounds. *Cancer Res. 50*, 5084–5087.

(156) Wargovich, M. J., and Goldberg, M. T. (1985) Diallyl sulfide: a naturally occurring thioether that inhibits carcinogen-induced nuclear damage to colon epithelial cells in vivo. *Mutat. Res. 143*, 127–129.

(157) Hu, P.-J., and Wargovich, M. J. (1989) Effect of diallyl sulfide on MNNG-induced nuclear aberrations and ornithine decarboxylase activity in the glandular stomach mucosa of the Wistar rat. *Cancer Lett. 47*, 153–158.

(158) Schaffer, E. M., Liu, J.-Z., Green, J., Dangler, C. A., and Milner, J. A. (1996) Garlic and associated allyl sulfur components inhibit *N*-methyl-*N*-nitrosourea induced rat mammary carcinogenesis. *Cancer Lett. 102*, 199–204.

(159) Wattenberg, L. W., Sparnins, V. L., and Barany, G. (1989) Inhibition of *N*-nitrosodiethylamine carcinogenesis in mice by naturally occurring organosulfur compounds and monoterpenes. *Cancer Res. 49*, 2689–2692.

(160) Wargovich, M. J., Imada, O., and Stephens, L. C. (1992) Initiation and post-initiation chemopreventive effects of diallyl sulfide in esophageal carcinogenesis. *Cancer Lett. 64*, 39–42.

(161) Hong, J.-Y., Wang, Z. Y., Smith, T. J., Zhou, S., Shi, S., Pan, J., and Yang, C. S. (1992) Inhibitory effects of diallyl sulfide on the metabolism and tumorigenicity of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in A/J mouse lung. *Carcinogenesis 13*, 901–904.

(162) Huber, W. W., McDaniel, L. P., Kaderlik, K. R., Teitel, C. H., Lang, N. P., and Kadlubar, F. F. (1997) Chemoprotection against the formation of colon DNA adducts from the food-borne carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in the rat. *Mutat. Res. 376*, 115–122.

(163) Suzui, N., Sugie, S., Rahman, K. M. W., Ohnishi, M., Yoshimi, N., Wakabayashi, K., and Mori, H. (1997) Inhibitory effects of diallyl disulfide or aspirin on 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine-induced mammary carcinogenesis in rats. *Jpn. J. Cancer Res. 88*, 705–711.

(164) Surh, Y.-J., Lee, R. C.-J., Park, K.-K., Mayne, S. T., Liem, A., and Miller, J. A. (1995) Chemoprotective effects of capsaicin and diallyl sulfide against mutagenesis or tumorigenesis by vinyl carbamate and *N*-nitrosodimethylamine. *Carcinogenesis 16*, 2467–2471.

(165) Tsuda, H., Uehara, N., Iwahori, Y., Asamoto, M., Iigo, M., Nagao, M., Matsumoto, K., Ito, M., and Hirono, I. (1994) Chemopreventive effects of β-carotene, α-tocopherol and five naturally occurring antioxidants on initiation of hepatocarcinogenesis by 2-amino-3-methylimidazo[4,5-*f*]quinoline in the rat. *Jpn. J. Cancer Res. 85*, 1214–1219.

(166) Goldberg, M. T., and Josephy, P. D. (1987) Studies on the mechanism of action of diallyl sulfide, an inhibitor of the genotoxic effects of cyclophosphamide. *Can. J. Physiol. Pharmacol. 65*, 467–471.

(167) Xie, Q., Liu, Y., Sun, H., Liu, Y., Ding, X., Fu, D., Liu, K., Du, X., and Jia, G. (2008) Inhibition of acrylamide toxicity in mice by three dietary constituents. *J. Agric. Food Chem. 56*, 6054–6060.

(168) Takada, N., Matsuda, T., Otoshi, T., Yano, Y., Otani, S., Hasegawa, T., Nakae, D., Konishi, Y., and Fukushima, S. (1994) Enhancement by organosulfur compounds from garlic and onions of diethylnitrosamine-induced glutathione *S*-transferase positive foci in the rat liver. *Cancer Res. 54*, 2895–2899.

(169) Guyonnet, D., Bergès, R., Siess, M. H., Pinnert, M. F., Chagnon, M. C., Suschetet, M., and Le Bon, A. M. (2004) Post-initiation modulating effects of allyl sulfides in rat hepatocarcinogenesis. *Food Chem. Toxicol. 42*, 1479–1485.

(170) Delker, D. A., Papanikolaou, A., Suhr, Y.-J., and Rosenberg, D. W. (2000) Diallyl sulfide enhances azoxymethane-induced preneoplasia in Fischer 344 rat colon. *Chem.-Biol. Interact. 124*, 149–160.

(171) Lin, M. C., Wang, E.-J., Patten, C., Lee, M.-J., Xiao, F., Reuhl, K. R., and Yang, C. S. (1996) Protective effect of diallyl sulfone against acetaminophen-induced hepatotoxicity in mice. *J. Biochem. Toxicol. 11*, 11–20.

(172) Wang, E.-J., Li, Y., Lin, M., Chen, L., Stein, A. P., Reuhl, K. R., and Yang, C. S. (1996) Protective effects of garlic and related organosulfur compounds on acetaminophen-induced hepatotoxicity in mice. *Toxicol. Appl. Pharmacol. 136*, 146–154.

(173) Fukao, T., Hosono, T., Misawa, S., Seki, T., and Ariga, T. (2004) The effects of allyl sulfides on the induction of phase II detoxification enzymes and liver injury by carbon tetrachloride. *Food Chem. Toxicol. 42*, 743–749.

(174) Manesh, C., and Kuttan, G. (2002) Alleviation of cyclophosphamide-induced urotoxicity by naturally occurring sulphur compounds. *J. Exp. Clin. Cancer Res. 21*, 509–517.

(175) Wattenberg, L. W. (1993) Prevention, therapy, basic science and the resolution of the cancer problem: presidential address. *Cancer Res. 53*, 5890–5896.

(176) Ioannides, C., and Lewis, D. F. V. (2004) Cytochromes P450 in the bioactivation of chemicals. *Curr. Top. Med. Chem. 4*, 1767–1788.

(177) Guengerich, F. P. (1992) Metabolic activation of carcinogens. *Pharmacol. Ther. 54*, 17–61.

(178) Brady, J. F., Ishizaki, H., Fukuto, J. M., Lin, M. C., Fadel, A., Gapac, J. M., and Yang, C. S. (1991) Inhibition of cytochrome P450 2E1 by diallyl sulfide and its metabolites. *Chem. Res. Toxicol. 4*, 642–647.

(179) Shu, L., and Hollenberg, P. F. (1997) Alkylation of cellular macromolecules and target specificity of carcinogenic nitrosodialkylamines: metabolic activation by cytochromes P450 2B1 and 2E1. *Carcinogenesis 18*, 801–810.

(180) Nims, R. W., and Lubet, R. A. (1996) The CYP2B Subfamily, in *Cytochromes P450: Metabolic and Toxicological Aspects* (Ioannides, C., Ed.) pp 136−160, CRC Press, Boca Raton, FL.

(181) Kim, D., and Guengerich, F. P. (2005) Cytochrome P450 activation of arylamines and heterocyclic amines. *Annu. Rev. Pharmacol. Toxicol. 45*, 27–49.

(182) Kawajiri, K., and Hayashi, S.-I. (1996) The CYP1 Family, in *Cytochromes P450: Metabolic and Toxicological Aspects* (Ioannides, C., Ed.) pp 77−97, CRC Press, Boca Raton, FL.

(183) Singh, S. V., Pan, S. S., Srivastava, S. K., Xia, H., Hu, X., Zaren, H. A., and Orchard, J. L. (1998) Differential induction of NAD(P)H: quinone oxidoreductase by anti-carcinogenic organosulfides from garlic. *Biochem. Biophys. Res. Commun. 244*, 917–920.

(184) Gelboin, H. V. (1980) Benzo[*a*]pyrene metabolism, activation, and carcinogenesis: role and regulation of mixed-function oxidases and related enzymes. *Physiol. Rev. 60*, 1107–1166.

(185) Mori, H., Sugie, S., Rahman, W., and Suzui, N. (1999) Chemoprevention of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine-induced mammary carcinogenesis in rats. *Cancer Lett. 143*, 195–198.

(186) Milner, J. A. (1996) Garlic: its anticarcinogenic and antitumorigenic properties. *Nutr. Rev. 54*, S82–S86.

(187) Guyonnet, D., Belloir, C., Suschetet, M., Siess, M.-H., and Le Bon, A.-M. (2002) Mechanisms of protection against aflatoxin B_1 genotoxicity in rats treated by organosulfur compounds from garlic. *Carcinogenesis 23*, 1335–1341.

(188) Jang, J. J., Cho, K. J., and Kim, S. H. (1989) Effects of allyl sulfide, germanium and NaCl on the development of glutathione *S*-transferase P-positive rat hepatic foci initiated by diethylnitrosamine. *Anticancer Res. 9*, 273–276.

(189) Jang, J. J., Cho, K. J., Lee, Y. S., and Bae, J. H. (1991) Modifying responses of allyl sulfide, indole-3-carbinol and germanium in a rat multi-organ carcinogenesis model. *Carcinogenesis 12*, 691–695.

(190) Takahashi, S., Hakoi, K., Yada, H., Hirose, M., Ito, N., and Fukushima, S. (1992) Enhancing effects of diallyl sulfide on hepatocarcinogenesis and inhibitory actions of the related diallyl disulfide on colon and renal carcinogenesis in rats. *Carcinogenesis 13*, 1513–1518.

(191) Matsuda, T., Takada, N., Yano, Y., Wanibuchi, H., Otani, S., and Fukushima, S. (1994) Dose-dependent inhibition of glutathione *S*-transferase placental form-positive hepatocellular foci induction in the rat by methyl propyl disulfide and propylene sulfide from garlic and onions. *Cancer Lett. 86*, 229–234.

(192) Wargovich, M. J., Jimenez, A., McKee, K., Steele, V. E., Velasco, M., Woods, J., Price, R., Gray, K., and Kelloff, G. J. (2000) Efficacy of potential chemopreventive agents on rat colon aberrant crypt formation and progression. *Carcinogenesis 21*, 1149–1155.

(193) Belman, S. (1983) Onion and garlic oils inhibit tumor promotion. *Carcinogenesis 4*, 1063–1065.

(194) EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). (2011) Scientific opinion on flavouring group evaluation 8, Revision 3 (FGE.08Rev3). *EFSA J. 9*, 1988.

(195) EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF). (2011) Scientific opinion on flavouring group evaluation 74, Revision 1 (FGE.74Rev1). *EFSA J. 9*, 1842.

(196) Beutler, E. (1994) G6PD deficiency. *Blood 84*, 3613–3636.

(197) Shults, W. T., Fountain, E. N., and Lynch, E. C. (1970) Methanethiol poisoning. Irreversible coma and hemolytic anemia following inhalation. *J. Am. Med. Assoc. 211*, 2153–2154.

(198) Iwamoto, F. M., Lamborn, K. R., Kuhn, J. G., Wen, P. Y., Yung, W. K. A., Gilbert, M. R., Chang, S. M., Lieberman, F. S., Prados, M. D., and Fine, H. A. (2011) A phase I/II trial of the histone deacetylase inhibitor romidepsin for adults with recurrent malignant glioma: North American Brain Tumor Consortium Study 03−03. *Neuro-Oncol. 13*, 509–516.

(199) Piekarz, R. L., Frye, R., Prince, H. M., Kirschbaum, M. H., Zain, J., Allen, S. L., Jaffe, E. S., Ling, A., Turner, M., Peer, C. J., Figg, W. D., Steinberg, S. M., Smith, S., Joske, D., Lewis, I., Hutchins, L., Craig, M., Fojo, A. T., Wright, J. J., and Bates, S. E. (2011) Phase 2 trial of romidepsin in patients with peripheral T-cell lymphoma. *Blood 117*, 5827–5834.

(200) Smith, B. L. (2000) Effects of low dose rates of sporidesmin given orally to sheep. *N. Z. Vet. J. 48*, 176–181.