Appendix C

Soil Fumigants—Exploring Potential Modes of Toxicological Interaction

Direct Chemical Reactions and Possible Interactions via Glutathione Depletion

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Introduction

Epidemiologic studies have sometimes revealed interactions between the effects of different toxicants in causing important health effects. Such cases include greater than additive interactions between cigarette smoking and either arsenic (Jarup and Pershagen, 1991; Pershagen et al., 1981) or asbestos exposure (Frost et al., 2011; Case 2006; Wraith and Mengersen, 2007) in increasing the risks of lung cancer.

These high profile examples, revealed by the relatively blunt tool of epidemiologic detection, give rise to the concern that there may be many more examples of interactions among the effects of different toxicants. This concern for the fumigants was one important reason to study them together. Absent extensive epidemiology however, we take the tack of exploring possible interactions from what we know mechanistically about the actions of these relatively potent toxicants.

For this first appendix we have selected two possible types of interaction because they lend themselves to at least tentative quantitative analysis from material we have assembled so far. The types of interactions explored below are

- Direct chemical reactions between metam sodium and the halogenated fumigants (chloropicrin and telone)

- Interactions via possible depletion of glutathione—a common cofactor in the metabolism of all three fumigants.

Theory and Past Experience on the Form of Interactions Among Risks From Induced by Other Carcinogens

The basic multistage model of cancer naturally gives rise to two different types of expectations for interaction depending on whether two agents primarily affect the same or different stages in the cancer process:

- Simple addition of risks is a natural expectation if the two agents affect the same type of transition—e.g. two "initiators" in the classic two-stage initiation/promotion model of cancer.
- Similarly, a multiplicative interaction of relative risks is the natural expectation if the two agents primarily affect different transitions in a two- or multi-stage cancer process.

Before proceeding with this analysis, however we should caution the reader not to expect major changes in anticipated risks except at relatively high doses, even if there is reason to expect the modes of action to be multiplicative in some cases. This can be illustrated theoretically:

Imagine that the risks of two chemicals in producing a specific effect have a multiplicative interaction—that is, R(combined) = R1 * R2. Consider three cases, in which the risks for each toxicant induce a 10% or a 100% increase in the incidence of the effect over the background that would occur in the absence of exposure.

For the 100% increase case, it is easy to see that we expect a doubling of the background risk for each toxicant, so

R(combined) = (2*background)*(2*background) = 4*(background)

This is, of course appreciably different--perhaps epidemiologically detectably so--from a simple additive case;

R(combined) = 2* background + background = 3*background.

Now, however, let us suppose that the doses of the fumigants are each10X lower than in the previous case, so that for the multiplicative interaction,

R(combined) = (1.1*background)* (1.1*background) = 1.21 * background.

This is barely distinguishable from an additive case:

R(combined) = background + .1* background + .1* background = 1.2 * background

So if we presume that most interactions are multiplicative or less than multiplicative, very special circumstances will often be required to produce epidemiologically detectable increases over background rates of disease unless the individual fumigants cause greater than a 10% increase in the incidence of some health effect.

It is worth asking whether both mechanistic understanding and past epidemiological experience sustains the assumption that most interactions will be multiplicative or less.

Complex Mechanisms

First, theoretical mechanisms do exist that could produce combined risks that are greater than would be expected from simple multiplication of the risks of individual carcinogens.

Imagine that one fumigant acts by alkylating DNA in a way that is normally very efficiently repaired. However despite this repair process, this first fumigant still causes a 10% enhancement of background tumor risk. Let us say that this type of DNA damage normally causes only 0.5% of the background tumor risk, but after exposure to the second fumigant the repair is inhibited by 20-fold leading to a 20*0.5% = 10% increase in the tumor risk with exposure to the second agent alone. In this case, if there is exposure to both agents at levels that separately enhance tumor risk by 10% we would get a total risk of Background

+ 0.1 * Background from 20-fold enhancement of the 0.5% of background from the inhibited type of DNA repair + 20*10% =2 X Background from the 20 fold enhancement of mutations from the first fumigant = 3.1 times the original Background tumor risk. This is of course larger than the 1.1 * 1.1 = 1.21 times background expected under a simple multiplicative risk model. In this way the interaction could be greater than would be expected from a simple multiplication of the risks from the individual fumigants. In addition, one could have a situation in which there is a basic multiplicative interaction from the two agents which operate at different stages in a multistage model, and additional enhancement of risk from the inhibition by one agent of the repair of the other agent's DNA adducts. This may be the case for the interaction of Hepatitis B virus with aflatoxin in enhancing liver cancer risk.

Past Epidemiological Experience

A superficial reading of the abstracts of many of the papers reporting interactions of risks among carcinogens cited above suggests observations that are intermediate between simple addition of risks and simple multiplication of the risks of the individual agents. However there is one substantial monograph from IARC (2004) that has extensive tables (reproduced as Tables 1 and 2 here) that report several observations of greater than multiplicative interactions of smoking and asbestos cancer risks. Of the data sets cited in these tables with classifications of interaction type in the final column, a plurality (7/16 = 44%) are listed as greater than multiplicative, whereas 5/16 are reported as multiplicative, and only a quarter (4/16) are reported as additive or intermediate. Overall, the impression given by this is that data sets with greater than multiplicative interactions are far from rare.

However examination of the first case of "greater than multiplicative" interaction [from the data of Marischnig et al. (1977) in Table 1] immediately gives rise to some suspicion of the classification. The relative risk reported for smoking alone is only 1.78—much smaller than the risk ratios reported for other data, and the comparison group is not non-smokers, but people who smoked as many as 14 cigarettes per day. Examination of the underlying data from this study provides the following relative risk observations for the smokers not exposed to asbestos:

.	.	Greatest number of cigarettes smoked daily			
.	0	1 to 14	15-24	25 or more	all smokers
Lung cancer	4	31	91	75	197
Controls	25	39	87	50	176
Relative risk	1	5	6.5	9.4	7.00
RR 95% CL		1.6-15.9	2.2-19.4	3.1-28.5	2.4-20.5

So it can be seen that mixing the 1-14 cigarettes/day group with the nonsmokers considerably dilutes the effect of smoking. Returning to the Marischnig et al. (1977) it

Table 1

IARC (2004) Compilation and Evaluation of Case Control Studies on the Combined Effects of Exposure to Asbestos and Smoking in the Causation of Lung Cancer

			Relative Risk				
Reference	Definition and source of asbestos exposure	Definition of smoking exposure	Not exposed to asbestos or smoking	Exposed to asbestos but not smoking	Exposed to smoking but not asbestos	Exposed to smoking and asbestos	Interaction*
Marischnig et al. (1977)	Yes vs no questionnaire on work history	>= 15 vs 0-14 cigarettes/day	1	1.08	1.78	5.57	>M
Blot et al. (1978, 1980, 1982)	Ever vs never worked in shipbuilding: interview of patients or proxies about work history	Current or former < 10 years					
		Georgia (n = 458)	1	1.28	4.71	7.58	~M
		Virginia (n = 319	1	1.88	3.09	4.87	~M
		Florida (n = 295)	1	1.8	6.01	7.79	blank
Rubino et al. (1979)	>=101 vs 100 fibre-years: work history, dust measurements	Smoker vs nonsmoker	0	0	1	2.32	blank
Pastorino et al. (1984)	Yes vs no: interview of patients or proxies about work history	>=10 vs 0-9 cigarettes/day	1	2.82	5.47	9.86	I*
Kjuus et al. (1986)	Heavy or moderate vs uncertain or none: interview of patients about asbestos exposure	>=10 vs 0-9 cigarettes/day	1	2.41	5.41	19.86	~M

Reference	Definition and source of asbestos exposure	Definition of smoking exposure	Not exposed to asbestos or smoking	Exposed to asbestos but not smoking	Exposed to smoking but not asbestos	Exposed to smoking and asbestos	Interaction*
Garshick et al. (1987	Yes vs no: work history	>50 pack-years vs never smoker; age < 65 years	1	1.2	5.08	6.82	blank
		age >= 65 years	1	0.98	9.14	8.96	blank
De Klerk et al. (1991)	High vs low: work history, dust measurements	Current or former < 10 years vs never-smoker or former > 10 years	1	2.24	3.44	9.57	>M
Minowa et al. (1991)	Definite or suspected vs none: interview of proxies about work history	Current or former < 10 years vs never-smoker or former > 10 years	1	not applicable because of zero division in odds ratio calculation	3.38	8.28	blank
Bovenzi et al. (1993)	Definite or possible vs none: interview of proxies about work history	Ever- vs never-smoker	1	1.83	10.13	15.89	blank
Gustavsson et al. (2002)	>= fiber-years vs none: reported work histories evaluated by an industrial hygienist and linked to workplace measurements	Current smoker vs never-smoker	1	10.2	21.7	43.1	blank

*These interaction conclusions are attributed to Saracci and Boffetta (1994) and Lee (2001). >M = greater than multiplicative interaction; M multiplicative; I intermediate between additive and multiplicative; A additive.
Source: IARC (2004).

Table 2

IARC (2004) Compilation and Evaluation of Cohort Studies on the Combined Effects of Exposure to Asbestos and Smoking in the Causation of Lung Cancer

Reference	Definition and source of asbestos exposure	Definition of smoking exposure	Reference Group	Not exposed to asbestos or smoking	Exposed to asbestos but not smoking	Exposed to smoking but not asbestos	Exposed to smoking and asbestos	Interaction**
Elmes and Simpson (1971)	Study group: inferred from the nature of population studied	Smoker vs nonsmoker	External	1	0 (only 5 nonsmokers at risk)	(7.13)*	112.94	blank
Selikoff and Hammond (1975)	Study group: inferred from the nature of population studied	Ever vs never-smoker	External	1	8.44	(7.13)*	73.71	(>M)
Hammond et al. (1979)	Study group: inferred from the nature of population studied	Ever vs never-smoker	External	1	5.17	10.85	53.24	M
Selikoff et al. (1980)	Study group: inferred from the nature of population studied	Ever vs never-smoker	External	1	25	(7.13)*	33.44	I**
Acheson et al. (1984)	Medium or heavy vs background: work history and dust measurements	Ever vs never-smoker	Internal	0	1	0	2.57	~M
			External	1	6.07	(7.13)*	15.53	blank

Reference	Definition and source of asbestos exposure	Definition of smoking exposure	Reference Group	Not exposed to asbestos or smoking	Exposed to asbestos but not smoking	Exposed to smoking but not asbestos	Exposed to smoking and asbestos	Interaction**
Berry et al. (1985)	Severe vs. low to moderate work history	Ever vs never-smoker (1960-1970)	Internal					
		Men		1	0	1.15	1.93	(>M)
		Women		0	1	0	2.26	(>M)
		(1971-1980)	External					
		Men		1	0	(7.13)*	19.33	(A)***
		Women		1	15	(7.13)*	33.97	(I**)
Hilt et al. (1985)	Exposed vs population controls: work history	Ever vs never-smoker	Internal	1	0	5.84	25.2	>M
Neuberger and Kundi (1990)	All workers: work history and dust measurements	Cigarettes/day smoked	Blank	blank	blank	blank	blank	blank
Hughes and Weill (1991)	Study group: work history and dust measurements	Ever vs never-smoker	External	1	0	(7.13)*	~13	blank
Cheng and Kong (1992)	Yes vs no: work history and dust measurements	Cigarette smoker vs nonsmoker	Internal	1	5.44	1.57	8.73	M
McDonald et al. (1993)	>= vs <60 million particles per cubic foot X years: work history and dust measurements	Ever vs never-smoker	Internal	1	1.65	4.46	4.51	blank
			External	1	4.07	(7.13)*	11.13	blank
Zhu and Wang (1993)	Yes vs no: work history and dust measurements	Smoker vs nonsmoker	Internal	1	3.78	1.83		blank
Meurman et al. (1994)	Heavy vs moderate: work history	Smoker vs nonsmoker	Internal	1	0.83	6.27	6.16	blank

			External	1	3.21	(7.13)*	23.87	blank
Oksa et al. (1997)	Study group: medical interview	Ever vs never-smoker	External					
		Asbestos sprayers		1	0	(7.13)*	74.77	blank
		Patients with asbestosis		1	0	(7.13)*	81.72	blank
		Patients with silicosis		1	0	(7.13)*	22.34	blank

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*Value assumed by Lee (2001) from the British Doctors' Study.

** These interaction conclusions are attributed to Saracci and Boffetta (1994) and Lee (2001). >M = greater than multiplicative interaction; M multiplicative; I intermediate between additive and multiplicative; A additive.

Source: IARC (2004).

becomes apparent that the likely reason this was done in the IARC monograph and other compilations is that Marischnig et al. simply do not provide the data for the lung cancer incidence in nonsmokers exposed to asbestos; they only provide the data for lung cancers in the aggregate of nonsmokers and smokers of 1-14 cigarettes/day.

A possible example of an apparently greater than multiplicative interaction* mediated in part by inhibition of a specific type of DNA repair is the interaction between aflatoxin and hepatitis B in causing liver cancer (Becker et al. 1998; Qadri et al. 2011). Hepatitis B produces a protein, the HBx, which specifically inhibits DNA excision repair. As it happens, this type of repair is the one primarily involved in dealing with bulky DNA adducts, such as those produced by aflatoxin. In a mouse model, expression of the HBx protein was associated with a significant shift in the spectrum of mutations induced by aflatoxin. Wild-type mice exposed to aflatoxin primarily showed increases in transition-type mutations,** but mice expressing HBx showed mainly induction in mutations mediated by DNA transversions (Madden et al. 2002).

The general issue of the mode of interaction observed in epidemiologic data for combined exposures to two human carcinogens is important enough that it deserves a closer and more quantitative analysis in which information from different data sets is statistically weighted and combined to reveal more rigorously what the data as a whole are actually saying. As it happens, one of the source papers for the classifications provided in these tables (Lee, 2001) provides a well-executed and well-documented example of such an analysis. These authors provide numerical counts of lung cancer cases and non-cases for those with and without both smoking and asbestos exposures. The overall conclusion is,

> "There was no overall departure from the multiplicative model, the proportional increase in risk of lung cancer with exposure to asbestos being estimated as 0.90 [95% confidence interval (95% CI) 0.67 to 1.20] times higher in smokers than non-smokers."

However the scope of the current project on fumigants does not permit an extensive effort

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* Data of Qian et al. (1994) reported in DHHS (2003) are:

Odds Ratios of Hepatocellular Carcinoma in Subjects Stratified Jointly by HBsAg Serology and Aflatoxin Exposure

	HBsAg(-)		HBsAg(-)	
Aflatoxin Urinary Metabolites	Cases/Controls	OR (95% CL)	Cases/Controls	OR (95% CL)
Negative	5/134	1	9/24	7.3 (2.2-24.4)
Positive	13/102	3.4 (1.1-10.0)	23/7	59.4 (16.6-212)

A simple multiplicative interaction would produce central estimate expected odds ratio of 7.3*3.4 = 24.8. The actual observed ratio of 59.4 is over twice this, but the expected 24.8 is still within the 95% confidence limits.

** DNA base substitution mutations are "transitions" if they involve interchanges of either one one-ring pyrimidine for another (e.g. Cytosine for Thymidine) or one two-ring purine for another (e.g. Adenine for Guanine). By contrast, "transversions" involve the substitution of a purine for a pyrimidine or vice versa.

to expand and reanalyze the database on which the Lee (2001) effort was based.

Direct Chemical Reactions Among the Fumigants

The halogenated fumigants chloropicrin and 1,3-dichloropropene have long been known to react with metam sodium via a straightforward SN2 nucleophilic substitution.

Because of this, some authors have considered these fumigants to be "incompatible" even though they are commonly marketed together for use in combined formulations (Zheng et al. 2004a). Zheng et al. provide the following rate constants for the reactions and 50% disappearance times in mixtures with 1 mM metam sodium at 21 degrees C at a near neutral pH of 6.9 (Table 3):

Table 3.
Second-Order Transformation Rate Constant and % Dissipation time (DT50) for Chloropicrin and 1,3-D (1 mM) in Aqueous Solution Containing an Equal Initial Concentration of Metam Sodium (1 mM) at 21 °C

fumigant	rate constant (per mM, per min)	DT50 (min)
chlorpicrin	1.73 +/- 0.05	0.58
cis-1,3-D	0.013 +/- 0.03	153.8
trans- 1,3-D	0.0039 +/- 0.0014	532

Source: Zheng et al. (2004a)

It is straightforward to convert these rate constants for chloropicrin and telone into expected rates of loss of the fumigant in the environment, given an assumption about how much metam sodium is likely to be present:

d[fumigant conc]/dt = k [metam sodium][fumigant conc]

d[fumigant conc]/[fumigant conc} = k [metam sodium}

Figure 7 of the Zheng et al. (2004a) paper shows the effects on fumigant concentrations of simultaneous vs sequential treatment with metam sodium at 0.5 mmoles/kg of soil. Assuming this is a reasonable estimate of likely in-use metam sodium concentrations we get the following expectations for the rate constants for degradation of chloropicrin and the telone isomers, and their corresponding half-lives in treated soil (Table 4):

Table 4.
Pseudo First Order Rated Constants and Expected Half-Lives for the Disappearance of Chloropicrin and Telone Isomers in Soil at 21 °C in the Presence of .5 mM Metam Sodium

	pseudo first order loss constant		T1/2	
chlorpicrin	0.865	per min	0.80	min
cis-1,3-D	6.50E-03	per min	107	min
trans- 1,3-D	1.95E-03	per min	355	min

From this it is apparent that nearly all chloropicrin mixed with .5 mM metam sodium will be converted to reaction products within an hour of application, and conversion of the telone isomers to reaction products will likely be appreciable. Particularly for the more rapidly-reacting chloropicrin, this raises a safety concern about how the reaction products compare the original chlorinated fumigants in toxic potency for different health effects. The initial product of the reaction of chloropicrin with metam sodium is dichloronitromethane, and subsequent dechlorination reactions yield monochloronitromethane and nitromthane (Zheng et al. 2004).

For the telone isomers, initial reaction with metam sodium yields an addition product, 1-chloro-3-dithiocarbamate-propene.* Loss of methyl isothiocyanate then yields a thiol (SH). Two molecules of this thiol can then connect to form a product with a disulfide linkange:

$ClCH=CH-CH_2-S-S-CH_2-CH=CHCl$

This compound retains the double bonds from the original telone which, like vinyl chloride, are likely to be converted to DNA reactive epoxides. Whether the resultant molecules (and the intermediate addition product) will have greater or lesser carcinogenic potency than the telone isomers is not yet known. This would be a natural issue for evaluation with future experimental work.

Possible Interactions via Depletion of Glutathione at High Exposures

Glutathione depletion is another candidate mechanism of interaction among exposures of the fumigants. Glutathione conjugation is frequently a step in the elimination of toxicants via the kidney. As can be seen in Figure 1, glutathione is a cofactor for the metabolism of all of the fumigants—with chloropicrin (trichloronitromethane) consuming at 2-4moles per mole of fumigant, and the other fumigants consuming one mole of glutahthione per mole of fumigant. If glutathione were to be appreciably depleted by reaction with one fumigant, this would lead to slower metabolism of the other fumigants, thus enhancing their opportunity to react with cellular macromolecules such as DNA and proteins.

* A Pubmed search for this compound yielded no results.

To assess the potential for this mechanism of interaction we need to understand how much glutathione conjugation must occur in order to cause appreciable depletion. The major site of systemic glutathione production is the liver. Recently the flux of liver glutathione synthesis has been measured in vivo in humans using an innovative magnetic-resonance imaging approach with 13C-labeled glycine by Skamarauskas et al. (2014). Based on measurements in a small group (3) of healthy young men, average hepatic glutathione synthesis is about 0.32 (+/– 0.18) mmoles/kg-hour. This compares with an average of 0.76 in the same units for rats. Communication with the corresponding author (Pete Thelwall) revealed that the "kg" referred to in this case are kg liver weight, not body weight. Further, the result is in terms of 13C-labled glutathione, not total glutathione, so to characterize overall glutathione synthesis we need to divide by the fraction of total glutathione that is 13-C labeled. Based on plasma glutathione measurements, this appears to be about 0.64. Multiplying by the average liver weight of young men (1.51 kg*) (Molina and Dimaio, 2012) and dividing by 0.64 yields a total flux of about 0.77 mmole of glutathione per hour.

In order to consume as much as 10% of this flux, it would therefore be necessary to have a fumigant intake that would react with about .077 mmole of glutathione per hour. For chloropicrin, which could consume as much as 4 moles of glutathione per mole of fumigant (Figure 1), we could begin to expect as much as 10% glutathione flux reduction with a continuous intake of about 0.019 mmole/hour.

The concentration of chloropicrin needed to provide this intake via inhalation can be readily calculated depending on the breathing rate. "1 ppm" means there is one mole of chloropircrin in a million moles of air. One mole of air at 25 °C occupies about 24.465 liters, or .02465 cubic meters. So 1 ppm of chloropicrin means that there is a millionth of a mole of chloropicrin--0.001 mmole--in that volume, that is, .001/.02465 = 0.0409 mmole/cubic meter.

Table 5 gives a detailed set of breathing rates for different adult age groups in units of cubic meters per hour. These were calculated from values in the 2011 version of the EPA Exposure Factors Handbook. Based on the average adult value for light activity (0.74 cubic meters/hour), the concentration of chloropicrin needed to deliver 0.019 mmole per hour is 0.019/(0.74*0.0409) = 0.64 ppm. For moderate intensity activity (1.59 cubic meters/hour), this falls to 0.29 ppm for the average adult, and about 0.22 ppm for an adult with a breathing rate at the 95th percentile for moderate intensity activity (2.15 cubic meters/hour).

These concentrations of chloropicrin are slightly larger than current guidance levels for chloropicrin exposure. The NIOSH recommended exposure limit is 0.1 ppm over an eight hour period (US Department of Health and Human Services 1995). For a 1 hour exposure, the AEGL-2 level for "disabling" effect is listed as 0.15 ppm, based on "severe ocular irritation and possible

* This is based on observations of an average liver weight of 1561 grams in 232 young men (average age 23.9, average weight 76.4 kg) who had died of head trauma, projected to the a model body weight of 70 kg.

respiratory effects in human volunteers (Reaves, 2006)" (USEPA, 2008). Therefore we can expect a relatively modest potential for interaction among the fumigants for adults via systemic glutathione depletion at levels far below those permitted on other bases.

Figure 1. Consumption of Glutathione (GSH) in the Metabolism of Trichloronitromethane (Chloropicrin), 1,3-Dichloropropene (Telone), and Sodium N-Methyldithiocarbamate (Metam Sodium)

Figure 2. Consumption of Glutathione (GSH) in the Metabolism of Trichloronitromethane (Chloropicrin), 1,3-Dichloropropene (Telone), and Sodium N-Methyldithiocarbamate (Metam Sodium)

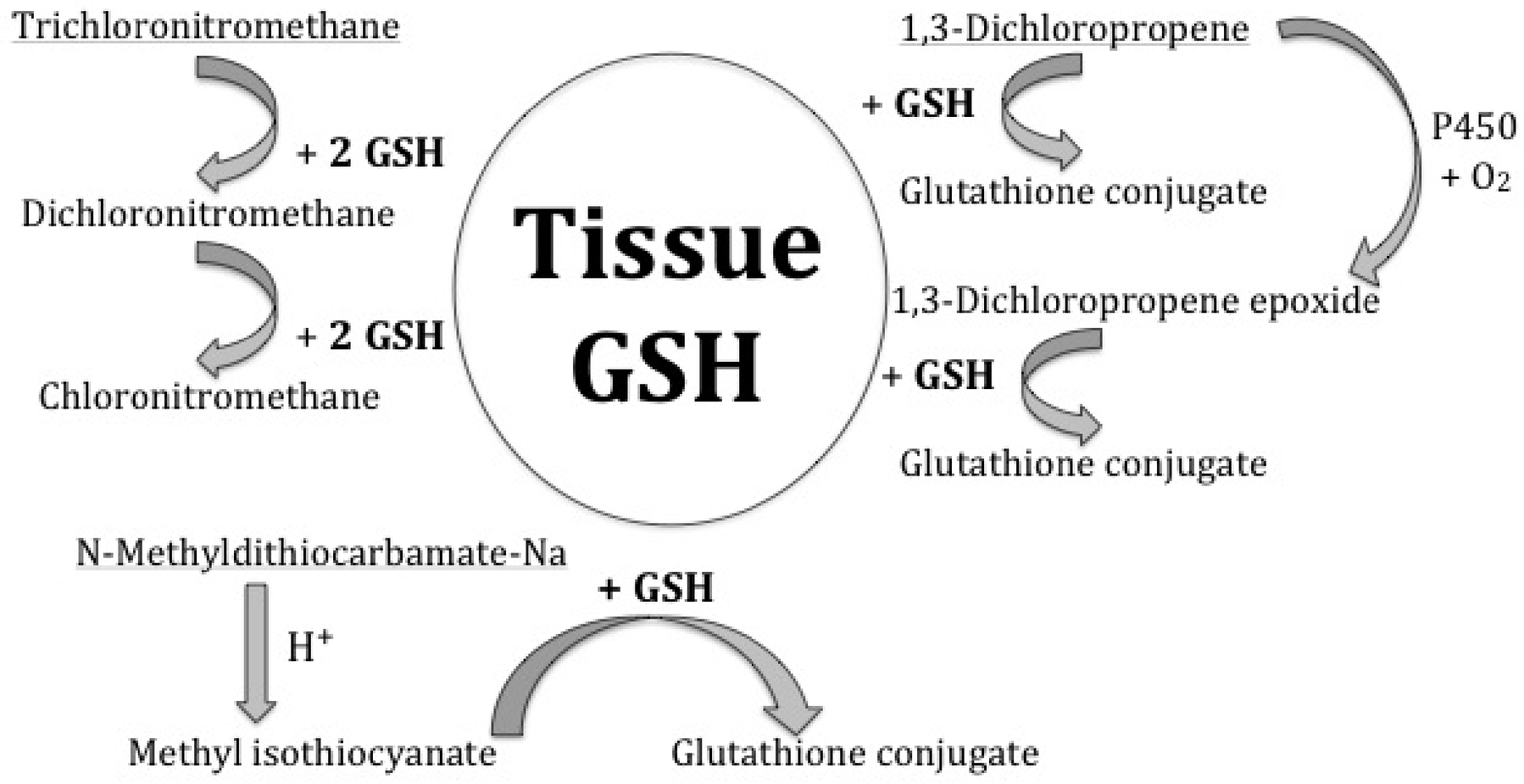

Table 5

Adult Breathing Rates in Cubic Meters/Hour, Based on Minute Ventilation Values in the 2011 EPA Exposure Factors Handbook

	Mean estimates of breathing rates			95th perecentile estimates of breathing rates		
Adult age groups (yrs)	Light intensity	Moderate Intensity	High intensity	Light intensity	Moderate Intensity	High intensity
21-	0.72	1.56	3	0.96	2.28	4.56
31-	0.72	1.62	2.82	0.96	2.22	4.32
41-	0.78	1.68	3.12	0.96	2.34	4.56
51-	0.78	1.74	3.18	1.02	2.4	4.68
61-	0.72	1.56	2.82	0.96	2.04	3.96
71-	0.72	1.5	2.82	0.9	1.92	3.9
81-	0.72	1.5	2.88	0.9	1.86	4.08
Average	0.74	1.59	2.95	0.95	2.15	4.29

This still leaves the possibility that there might be more significant glutathione depletion locally in respiratory tissues where chloropicrin and the other fumigants are absorbed. The most extensive set of modeled normal glutathione generation and loss rates appears in an older pharmacokinetic model of ethylene oxide metabolism (Hattis, 1987). These estimates were based on glutathione levels in different rat tissues, and observations of glutathione concentration decline after administration of an inhibitor of glutathione synthesis (buthionine solfoximine) (Griffith and Meister, 1979). The basal estimates of glutathione concentrations were taken unchanged from the rodent observations, but the synthesis rates were allometrically scaled with the approximately 0.25 power that describes relative rates of metabolism across species. [Human metabolism rate = rat metabolism rate * $(.25/70)^{0.25}$ = rat metabolism rate * .244.] Table 6 gives the basal concentrations and normal loss rates in different tissues inferred in this way for this model, aggregated into standard organ groups used for pharmacokinetic modeling.

Table 6

Inferred Baseline Levels and Normal Metabolic Loss Rates for Glutathione in Different Human Tissues—Model of Hattis (1987)

	Tissue weight (kg)	mmoles GSH/kg tissue	GSH stock (mmoles)	Fract normal metabolic per hour	Total GSH Flux Expected (mmole per hour)
Lung	0.464	1.025	0.476	1.2	0.57
Liver	2.476	4.24	10.55	0.037	0.39
Muscle group	34.756	0.75	26.1	0.021	0.54
Vessle-Rich Group (other than Liver)	3.551	1.84	6.53	0.18	1.18

An early check on the implications of this model is to compare the GSH flux estimate for the liver in this model (.39 mmole/hour) with the value of about 0.77 mmole/hour derived from direct observations in the three people reviewed earlier in this section. The older model estimate is within two-fold of the new observation. Given the limited sample size of the new observation, and the uncertainty in the other assumptions that went into the calculation (e.g. use of the fraction of C-13 glutathione in the plasma to estimate the fraction of total glutathione with this label in the liver) we think this tends to support using the estimates from the older model as a preliminary basis to evaluate the potential for glutathione depletion in the lung. As it happens the GSH flux estimates for the liver and lung are similar, leading to an expectation that there will be similar potential for glutathione depletion in the two organs. A further caveat to this is that the projections for human lung glutathione do not reflect the substantial amount of local oxygen usage, and therefore generation of reactive oxygen species, that would be expected for higher levels of activity during farm work. Such an elevated baseline of oxidative burden during activity has yet not been directly studied, to our knowledge, but it could somewhat enhance the vulnerability of respiratory tissues to the kind of oxidative stress produced by the three fumigants.

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