Pulmonary toxicity associated with occupational and environmental exposure to pesticides and herbicides

Aalt Bastab,b,c, Khrystyna O. Semenb and Marjolein Drentb,c,d

Purpose of review
Critical review on the notion that exposure to pesticides and herbicides lead to adverse effects in pulmonary health.

Recent findings
The lung effects of several chemical classes of pesticides and herbicides is biologically plausible. However, the studies that describe the association between exposure and toxic lung effects have numerous limitations. Critical evaluation of the studies that are performed shows that assessment of occupational or environmental exposure to pesticides and herbicides is cumbersome. Moreover, the health effects are not always clearly established due to the use of questionnaires and self-reported data instead of lung function measurements or diagnostic work-up by physicians.

Future studies should preferably better characterize the exposure. Genetic phenotyping should be included to understand and strengthen possible (individual) associations between exposure and health outcome. It should be realized that combined exposure to multiple environmental chemicals may lead to different health effects than exposure to individual chemicals.

Summary
The relation between exposure to pesticides and herbicides and lung toxicity is less clear than generally assumed. Adverse lung effects seem multifactorial and needs further research. Preventive measures remain key.

Keywords
asthma, chronic obstructive pulmonary disease, environmental, glyphosate, herbicides, meta analysis, organophosphate, paraquat, pesticides

INTRODUCTION
Several critical systematic reviews have been written focusing on the association between pesticide and herbicide exposure and prevalence and incidence of airway disease [1,2,3,4]. The fear of the general public for man-made chemicals, so-called chemophobia, seems to narrow down to pesticides/herbicides sprayed during crop production. It has been suggested that we will go to great lengths to avoid these chemicals, disproportional to the actual danger posed when used correctly [5]. The advantage in harvest yield and frequently harvest quality when using pesticides/herbicides seems for many consumers not to outweigh the fear. In addition to that, classical toxicological studies are rather easy to conduct because these chemicals are readily available in pure form and in large quantities.

Moreover, several classes of pesticides/herbicides have toxicological properties that make adverse associations between pesticide/herbicide exposure and lung diseases biologically plausible. However, epidemiological evidence for a causal relation between pesticide/ herbicide exposure and respiratory distress is much more difficult to establish. The aim of this review was to present what is known about pulmonary toxicity associated with exposure to pesticides and herbicides.

*Maasstricht University Campus Venlo, Venlo, †Department of Pharmacology and Toxicology, FHML, Maastricht University, Maastricht, ‡ILD Care Foundation Research Team, Ede and §Department of Pulmonology, ILD Center of Excellence, St. Antonius Hospital, Nieuwegein, The Netherlands Correspondence to Aalt Bast, Maastricht University, Campus Venlo, P.O. Box 8, 5900 AA Venlo, The Netherlands Tel: +31 43 3883195; e-mail: a.bast@maastrichtuniversity.nl

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BIOLOGICAL PLAUSIBILITY
Categorizing pesticides shows that there are chemical classes that can be predicted to affect lung function because of their molecular mechanism of action.

Inhibition of acetylcholinesterase
Organophosphate and carbamate pesticides inhibit acetylcholinesterase. Organophosphates [6] are usually esters, amides or thiol derivatives of phosphoric acid. The \( R_1 \) and \( R_2 \) groups are mostly alkyl or aryl groups. When linked to phosphorus via an oxygen atom the compound is called a phosphate (Fig. 1A). Bonding via a sulfur is a phosphorothiolate (Fig. 1B). Compounds in which the phosphorus is linked via a double bond to sulfur are termed phosphorothioates (Fig. 1C). Compounds depicted in Fig. 1D, phosphoramidates, have a carbon atom linked to phosphorus via an NH-group. The X-moiety in organophosphates can be substituted or branched aliphatic, aromatic, or heterocyclic group linked to the phosphorus atom via an –O– or –S– which makes the compound more labile. The lability of the bond between the so-called leaving group X and the phosphorus atom in the organophosphatase determines reactivity with acetylcholinesterase and forms a phosphoryl-acetylcholinesterase complex.

Carbamate pesticides form a carbamyl-acetylcholinesterase complex, thus inhibiting acetylcholinesterase. Inhibition of acetylcholinesterase by either organophosphates or carbamates gives accumulation of acetylcholine at parasympathetic neuroeffector junctions (muscarinic effects in the lungs) and autonomic ganglia (nicotinic effects in the brain) leading to central nervous system (CNS) effects (Fig. 2).

The muscarinic \( M_3 \) receptor effects may lead to hypersecretion of mucus (cough) and a contraction of bronchial smooth muscles. The lability of the carbamyl-acetylcholinesterase complex compared to the organophosphate complex limits the value of cholinesterase activity measurement in blood as a diagnostic index of carbamate poisoning. It also accounts for the lower symptom-producing and lethal doses that exist in case of poisoning with organophosphate compounds compared to carbamates. Children or persons with preexisting conditions such as asthma or chronic obstructive pulmonary disease (COPD) are expected to be more vulnerable.

Reactive oxygen species
Laboratory research has shown that organohalogen pesticides such as dieldrin and lindane may cause the production of reactive oxygen species, which has been implicated in the immunotoxicity of these compounds via neutrophilic activation [7]. It has been suggested that environmental organochlorine insecticides may lead to chronic activation of monocytes and thus to pathogenic oxidative stress and

KEY POINTS
- Pesticides and herbicides can lead to pulmonary complications such as interstitial lung disease, asthma and COPD.
- Conclusions of epidemiological studies are based on limited data.
- Toxicity is multifactorial, depending on dose, age, circumstances and genetic predisposition.
- Appropriate protection is strongly recommended, as exposure is not harmless.
- Future studies should focus more on pathophysiological processes and therapeutic options.
inflammation [8]. Reactive oxygen species play a crucial role in the occurrence of several lung pathologies. An almost forgotten phenomenon is that the relaxing smooth muscle beta-adrenoceptor system seems to be more sensitive for damage by oxidative stress than the constricting muscarinic receptor system. The resulting disbalance in favor of the muscarinic receptor is important in the overall bronchospasm after oxidative stress [9].

Organophosphates have also been reported to inhibit the parasympathetic prejunctional muscarinic M2 receptor function [10,11] leading to airway hyperreactivity.

A special case is the herbicide paraquat. The use of this compound has been prohibited in the European Union. In other parts of the world, it is still a commonly used herbicide [12]. The Environmental Protection Agency in the USA classified paraquat as ‘restricted use’. This means that it can be used by licensed applicators. Paraquat is a so-called redox cycling compound that generates superoxide anion radicals. The oxidative stress leads to very severe lung damage and may cause pulmonary fibrosis. Recently it has been reported that paraquat disrupts the anti-inflammatory action of cortisol in human macrophages [13]. The anti-inflammatory action of cortisol might thus become hampered. Similarly, it may limit the effectiveness of a corticosteroid treatment after a paraquat poisoning.

**Systematic Reviews**

Several systematic reviews have assessed the effect of pesticide exposure on respiratory health [1,2,3**,4]. The first two meta analyses [1,2] included articles up to the end of 2013. The paper by Ratanachina et al. [3**] used several new epidemiological studies (up to October 2017) describing associations between pesticide exposure and lung function. The last literature review dates from 2020 [4]. The conclusions of the study by Doust et al. [1] were that the results were suggestive of associations between pesticide exposure and an increased likelihood of wheezing symptoms and asthma, with the evidence being stronger for an association in children than in adults. In contrast, the overall body of evidence was much weaker for an adverse association between pesticide exposure and an increased likelihood of COPD, with the symptomatic data being finely ambiguous and a notably paucity of studies utilizing objective lung function data that is critical for the diagnosis of COPD.

General problems were noted in the meta-analysis of Doust et al. [1]. Most of the included studies relied on questionnaires to typify likely exposures. This is of course vulnerable to recall bias and exposure misclassification. Self-reported exposure can easily entail nonrealized co-exposure to other respiratory hazards. Possibly the effect of a pesticide might in reality be the adverse effect of a co-formulant(s) present in commercial pesticide preparations. Exposure to multiple agents can occur which at best leads to potential associations. Failure to adjust for socioeconomic status as in many of the cited studies, likely introduces a systematic bias in favor of an association between pesticide exposure and asthma in adults. Further comments that restrict the value of the meta-analysis are that only very limited pulmonary function measurements were done in the included studies. Only a few

**Immunological alterations**

Several studies have suggested that exposure to pesticides favors the T-helper cell (Th) type 2 immunophenotype associated with asthma and allergy. Early studies on mice showed that exposure to low levels of the organophosphate pesticide malathion showed higher levels of degranulated mast cells than control mice [14], indicative for a type 1 allergic reaction. There are several indications in children that potentially (vide infra) are exposed to organic phosphates have enhanced Th2 activity and experience higher rates of asthma and wheezing [15]. The continuous debate on the toxicity of glyphosate was recently broadened by discussions on the effect of the herbicide on the immune system [16].

Organophosphate and organochlorine pesticides increase the allergenic potential of environmental chemical allergens in Balb/c mice by inducing an increased surface antigen expression of T cells [17].
studies used biomonitoring or employed biological effect monitoring. Moreover, publication bias could not be tested [18]. In the critical evaluation by Doust et al., it was concluded that high-quality research is needed to demonstrate causality which also considers co-exposure confounding (also by nonpesticides) and other factors.

The conclusion of the study by Mamane et al. [2] was that occupational exposure to pesticides presents a risk to the respiratory tract such as the development of asthma or chronic bronchitis. They could not conclude that occupational exposure presents a risk of COPD.

An important caveat to draw any conclusion about toxic effects to the respiratory tract is that only a few studies measured lung function parameters. In some studies, symptoms or diseases were self-reported by subjects. It was emphasized by the authors that it would be preferable to use validated questionnaires from lung specialists in the diagnostic workup in a standardized fashion and standardize lung function tests. It was further highlighted that in order to in order to be able to determine a causal association, criteria such as the strength of the association, consistency, specificity, temporality, dose-response link, biological plausibility, experimental evidence and analogy should be met. Many of the reviewed studies did not meet these criteria.

The recent meta-analysis by Ratanachina et al. [3], reviewed the literature on the pulmonary effect of occupational and environmental pesticide exposure taking into account lung function parameters that were appropriate. The conclusion was that there is tentative evidence that that occupational exposure to acetylcholinesterase inhibiting pesticides reduces Forced Expiratory Volume after 1 second/Forced Vital Capacity (FEV1/FVC) among farmers. It was further emphasized by the authors that high heterogeneity exists between studies with regard to exposure assessment and the adjustment for potential confounders. It was also suggested that larger studies are needed that apply more accurate exposure assessment and studies on preventive strategies. Moreover, respiratory surveillance should be enhanced in workers exposed to pesticides.

In the recent literature review by Tarmure et al. [4], it was concluded that exposure to pesticides is highly correlated with respiratory pathologies like asthma, COPD and cancer. It was emphasized that contact with pesticides occurs in the production, transport, preparation or application phase. The limitations of the reviewed literature were however less well described in this report.

It is clear from the discussion of these meta-analyses that the current main limitations are definition of the health effect and the assessment of exposure. In the meta-analyses [1,2,4], the health effect is based on either self-reported data or the diagnosis of a physician, but not on measures of lung function or bronchial responsiveness. The exposure concerns frequently a mix of active ingredients, the concentration is mostly unknown and the duration of exposure is frequently not identified. Important criteria to be able to determine a causal relationship between pesticide exposure and pulmonary health effect is therefore difficult to establish.

**INDIVIDUALIZATION**

To really understand the possible effect of pesticide exposure on respiratory health not only the exposure should be described in detail (*vide supra*). We should also take into account that individual vulnerability to various lung diseases exists. Multiple subtypes and phenotypes exist, such as childhood onset allergic asthma, adult onset atopic asthma, adult onset nonatopic asthma, hypereosinophilic asthma or asthmatic patients with granulomatosis. Moreover, variants in several genes are associated with increase vulnerability to asthma [19]. In COPD the disease is the result of genetic susceptibility and environmental exposure [20]. It is however thus far not known, how pesticides influence genes that play a critical role in the susceptibility of asthma or COPD. Other respiratory diseases besides asthma and COPD, have also been linked to occupational pesticide exposure such as lung cancer [21,22] and pulmonary fibrosis [23]. Incidental reports on for example pulmonary thrombosis [22], or sarcoidosis [24] associated with pesticide exposure have been published. It is increasingly realized, that although population-based toxicology or pharmacology is of importance we would now gain more insight by molecular or genetic phenotyping in new studies.

**MECHANISM OF TOXICITY**

The molecular mechanism of the toxicity of paraquat that may lead to lung fibrosis is well understood. The herbicide paraquat undergoes redox cycling, i.e. the compound is reduced in a single electron process. The electron is subsequently shuttled to oxygen which results in a superoxide anion radical ($O_2^\cdot\cdot$). Upon dismutation $O_2^\cdot\cdot$ is converted to hydrogen peroxide which in the presence of iron ($Fe^{2+}$) can be transformed into the very reactive hydroxyl radical ($OH^\cdot$). Hydrogen peroxide can be reduced to water by the enzyme glutathione (GSH) peroxidase which uses GSH. In this reaction, GSH is oxidized and the oxidized form of GSH, glutathione disulphide (GSSG) is produced. GSSG is reduced to
GSH by glutathione reductase which acquires its reducing equivalents via the pentose phosphate pathway. In this pathway glucose-6-phosphate dehydrogenase (G6PD) plays a decisive role [25]. It can thus be understood that G6PD should be intact to protect against the reactive oxygen species that arise like in the process of redox cycling. Several redox cycling drugs are to be avoided by people with a G6PD deficiency [25]. An illustrative example in this respect is nitrofurantoin which is used to treat urine tract infection or primaquine used in the malaria. Similarly, it is to be expected that people with a G6PD deficiency are also more sensitive to lung toxicity associated with paraquat. More focus on genetic predisposition in explaining individual susceptibility to paraquat toxicity is warranted [25]. Many rapid tests are available to screen for G6PD deficiency [26]. It is easy to apply these tests for paraquat workers. A recent suggestion to treat paraquat poisoning with endogenously formed antioxidants, e.g. formed from creatinine, so-called ‘intrinsic antioxidants’ is very promising [27*].

It has also been suggested that in elderly individuals plasma levels of persistent organic pollutants (POPs) are associated with markers of increased oxidative stress thereby suggesting that even low dose background exposure to POPs may be involved in oxidative stress [28]. It is tempting to speculate the combination of paraquat with either genetic predisposition (G6PD deficiency) or co-exposure to other oxidative stress evoking compounds may aggravate the paraquat lung toxicity.

Fukuyama et al. [17] also suggested that simultaneous exposure to multiple (environmental) chemicals aggravates allergic airway inflammation more than exposure to individual chemicals. The combined toxicity may be affected by age, chemical structure, receptor binding and immune pathways involved [29]. The evaluation of combinatorial immunotoxic effects when conducting assessments of the safety of environmental or occupational chemicals needs further study.

We also showed that combination of exposures might lead to unexpected pulmonary effects. A good example is the case report of a patient who developed acute eosinophilic pneumonia associated with exposure with glyphosate-surfactant in combination with smoking [30]. The case highlights the importance of a thorough exposure history. The literature that has been grouped together several times over the years to make good overviews, sometimes provide strong conclusions. Critical evaluation of the literature however reveals several limitations with regard to characterization and quantification of health outcome as well as exposure assessment (Table 1).

### Table 1. Difficulties of assessment of exposure in performed studies

- Use of self-reported data or questionnaires
- No biomarkers
- Exposure to complex mixtures [instead of specific pesticide]
- Concentration of exposure is missing
- Time of exposure is missing
- Age race and gender as well as comorbidity may influence the effects
- Mostly nonprospective design

### CONCLUSION

Classical mechanistic and animal toxicological studies with pesticides and herbicides are relatively easy to perform because the compounds are available in pure form. Biological plausibility of the human toxicity can effortlessly be based on these data. However to draw definitive conclusions on whether pesticides/herbicides use have an effect on human pulmonary health is still difficult. The toxicity partly depends on the genetic vulnerability of an individual. This also explains the phenotype of the disorder.

It should be emphasized that future studies should preferably better characterize the exposure and health outcome, e.g., via standardized diagnostic work-up including pulmonary function tests. Genetic phenotyping of the participants with a certain disease should preferably be included to understand and strengthen possible associations between exposure and health effects. It would be preferable to perform studies with specific chemical groups of pesticides rather than with complex mixtures in one study to stimulate more clear and consistent conclusions.

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### Conflicts of interest

There are no conflicts of interest.

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- of particular interest
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