Considerations at defining Critical Levels for NH_3.

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1. Introduction[3]

The process of arriving at generally accepted, scientifically reliable quality standards for pollutants follows a pathway that covers several decades. The derivation of a Critical Level (CLE) for NH_3 is not an exception.

In a first phase, quality standards for pollutants that aim at protecting ecosystems are largely based on the No Observable Effect Concentration (NOEC) of the most sensitive species tested. This NOEC will decrease over years as the result of discovering higher sensitivities.

In the second phase, with continued investigation, a set of effect data comes available that allows evaluation of inter species variability. Specifying the protection level and reliability of a standard becomes possible.

Protecting species is not the only goal of standards. Protecting functioning of the system is another. NOECs for both goals and understanding causal relations between the two are needed, to decide which one is most adequate for a quality standard. Probably, the CLE for NH_3 is still in its first phase. But it has clearly possibilities to enter the second phase (see annex 1).

Section 1 deals with the types of responses on NH_3 and Section 2 with methods to convert information on response (or NOECs) into general effect thresholds (or CLEs).

An important issue in this workshop should also be to discuss the need of validation. To which degree should input data be validated? Peer refereed, other reports and chapters, reproduced by other teams, or a reliability based on judgement of recognized experts? It may be noted that the CLRTAP reviews on empirical critical loads for nitrogen deposition have integrated all of these sources of information (e.g. Achermann and Bobbink, 2003). Similarly, it can be asked what can field data mean for verification of critical levels? Surveys in the neighbourhood of ammonia sources can be very relevant, both for discovering new effects, as well as to estimate the consequences of long term exceedance of the CLE (see Annex 2).

Annex 3 and 4 inform the discussion that seems unavoidable in this workshop, on comparing CLEs and CLOs. Annex 3 compares the practical differences between the existing CLEs for NH_3 and CLOs for nitrogen. It may be argued that many of these differences are historical, reflecting different perspectives of the contributing scientists, and that there is an ongoing need to harmonize the application of the CLE and CLO approaches. In Annex 4 the relevance of distinguishing different N compounds is discussed.

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[3] This paper uses some abbreviations. CLE stands of Critical Levels and CLO for Critical loads. NOEC is the abbreviation for No Effect Level.

2. Relevance of responses

NH_3 acts as a macro-nutrient for all bio-systems. At low N-status, most plants respond on exposure to NH_3 with increasing their biomass production. With higher level of NH_3 exposure, biodiversity goes down, some species develop injury, the N circulation in the system accelerates and the system starts leaching N.

One of the challenges in this workshop is to judge which responses can be assumed to be adverse. In this section some considerations are presented.

2.1 Measurable N uptake.

With low atmospheric NH_3 concentration the "compensation point" of the foliage determines whether NH_3 will be taken up or not. In principle, this is the lowest relevant "NOEC". A CLE below this NOEC does not make sense. A plant emits NH_3 if the atmospheric NH_3 concentration is below its compensation point.

The compensation point of plant communities increases with higher and prolonged ammonia exposure (e.g. Sutton et al. 1995a). This well established for higher plant species, but less certain for lower plant species. Although such an increase is an effective adaptation by the plant itself, from the environmental point of view this phenomenon could it considered to be an indicator that the habitat is adversely affected. Moreover, re-emission means that this NH_3 hops over 10s of kms until it finds its sink in plants or vegetation with a really low compensation point.

Practical problems with using the compensation point to define a lowest NOEC are that it is not easy to measure and that it is variable (e.g. highly dependent on temperature and on N status of the plant), while the overall "canopy compensation point", integrating stomatal, leaf surface and ground surface exchange may be different to the "stomatal compensation point" (Sutton et al. 1995a,b). These interactions mean that a mixed plant canopy may contain a broad mix of compensation points for different species, which also vary strongly in space and time. Thus, while it can be concluded that the lowest NOEC will be larger than zero, it is not trivial to generalize the relevant values from NH_3 compensation point concentrations.

The foliar N content can easily be determined, and in forestry a N content of 2% is considered to be a threshold for increased stress sensitivity in trees, although not well validated[4]. Other indicators like foliar N/K are assumed to be even better. Of course, as long as the relation between N content and stress sensitivity is not fully explored, the debate will continue on whether it should be 1.5% or 1.8% or species dependent. The same applies to the 1.2% limit for Sphagnum, which is assumed by many as an effect threshold, although it still needs rationalised and verified appropriately. Hence while overall relationships are agreed, quantitative limits remain a matter of discussion. Similarly, it is well established that enhanced foliar uptake of N reduces N uptake by the roots (*e.g.* Srivastava & Ormod 1986).

2.2. Biochemical response

Several biochemical responses can be measured as a result of exposure to atmospheric N. For most plant species, foliar NH_3 and NH_4^+ are toxins. If a plant has sufficient detoxification capacity it will be converted into glutamine, arginine and other amino acids. Increased

[4] experiments with *Calluna vulgaris* by Sheppard & Leith (2002), aimed to validate this assumption, did not find a clear relation.

contents of those organic compounds or increased GS activity are indications for detoxification. Conversely, elevated foliar NO_3^- is not toxic. It suggests an excess N that is waiting to be incorporated in the N metabolism of the plant. Enhanced foliar Nitrate Reductase (NR) activity indicates this incorporation.

2.3. Morphology of the plant

Atmospheric N, especially NH_3 can cause erosion of the cuticular wax layer. In field studies, Kupcinskiene (2001) showed significant effects at concentrations below 23 µg m^{-3}. The relevance of wax erosion for vitality of the tree, however, is poorly understood (Thijse & Baas 1990).

Atmospheric NH_3, being a fertiliser generally causes thinner leaves (increased Specific Leaf Area (SLA); resulting in more evaporation and more uptake capacity for air pollutants) and increased shoot /root. This provides one mechanism by which NH_3 can increase the sensitivity of plants to drought.

Foliar injury is generally considered to be an adverse effect. Although it does say something about the aesthetics, it has no direct relation with the vitality of the plant. (i.e. several studies have shown that visible pollutant injury is not necessarily correlated with long term performance).

With prolonged elevated N input many tree species loose their apical dominance earlier (*e.g.* at an age of 25 instead of 50).

2.4. Reduced life span

It is general knowledge that with higher nitrogen input (including NH_3), woody species like trees and heath speed up their growth and biomass production, but also reach their maximum life span in fewer years. For forestry plantations that will generally not be a problem, because it probably has no negative impact on wood production per hectare. However, for *Calluna* the life span might go down from 40 to 10 years (Berendse, personal communication), possibly resulting in faster rejuvenation and higher costs of maintenance of such habitats.

2.5. Adaptation?

There are indications that vegetation can adapt to higher input of N without major ecological damage. In field experiments heathland in Scotland (and Scandinavian countries) seem to respond at much lower N exposure levels than heathland in the Netherlands (and Germany). This might be explained by adaptation over decades, for instance by selection of NH_x tolerant ecotypes. However, it may also be an artefact due to an inappropriate control treatments (i.e. with large background concentrations used in many chamber studies). The difference may also reflect the fact that, with lower background N deposition and NH_3 concentrations in Scotland and Scandinavia, it becomes possible to detect effects at lower levels. This would imply that in the Dutch and German field experiments the control treatments were also impacted by NH_3 and N deposition.

Needle fall is a common phenomenon in the neighbourhood of N-sources. Although this effect was a major indicator in the era of concern on forest decline, it can also be considered to be an effective adaptation to avoid too much N uptake.

2.6. Change in reproduction and stress tolerance

Going from low to higher N exposure, vegetation with a low N status will response first with increase in growth, but with higher exposures a decrease in reproduction and stress tolerance will occur. For example, in open top chamber (OTC) studies Leith et al. (2001) found NH_3 exposure to reduce flowering of *Eriophorum vaginatum*, although overall, growth of this species benefited from NH_3 treatment compared with other moorland species. The same results were found in *Antenaria dioica* and *Arnica Montana* (Van der Eerden 1992).
Again, the question is: should any response, the optimum or only decreases be considered to be adverse?

2.7. Soil and bark chemistry

Increased N input in the soil generally leads to increased microbiological activity, increased nitrate and ammonium concentration in edaphic ground water, increased N immobilisation in litter and increased output of N_2 N_2O and NO etc. All these features are signs of adaptation of the system itself, partly to the cost of the surrounding environment. Obvious adverse effects are, of course, exhausting the buffer capacity, acidification and leaching of nitrate to deeper ground water or surrounding surface water.

In the scope of CLEs, increasing evidence is developed that epiphytic lichens are particularly sensitive to NH_3 (Van Dobben & Bakker 1996, Wolseley et al., 2006, Frati et al., 2006). A gradual change in bark pH is probably more determining the effect that a direct uptake (Van Dobben & Ter Braak 1998), although this remains a topic of debate. For example, Sutton et al. (2006) found a significant additional effect of NH_3 to the effect via bark pH, based on analysis of a UK-wide survey of lichens on twigs and trunks.

2.8. Categorisation of NH_3 effects

CLEs for NH_3 can serve several applications (e.g. impact assessment of individual sources, protecting a specific ecosystem, environmental policy or biodiversity protection on the national or EU scale etc.). The type of effects that are considered to be adverse are dependent on this context. For instance, to monitor the productivity of a forestry plantation requires other assessments than the decision on extension of a farm or the protection of biodiversity of an adjacent moorland. The following table is a first attempt to categorise the effects mentioned above (Table 1).

In Table 1, the extent to which some of the risks mentioned can be translated into actual damage depends on ongoing improvement in the inter-calibration between responses. For example, we need to know better which levels of increase in foliar N or amino acid contents are benign for different ecological responses. Hence, a specified small increase in foliar N content of moss ground flora may indicate a level of excess N deposition or NH_3 that is not a significant threat to tree health, but may indicate a significant threat to lichen biodiversity.

Table 1. Categorisation of effect types in relation to their relevance for ecosystem functioning.

Risks		*Proved adverse effects*		
Evidence for a causal relation with a relevant endpoint on vegetation or ecosystem level is relatively low	*Causality is clear, but risk on adverse effects is probably low*	*On plant aesthetics*	*Directly or indirectly on plant growth or stress tolerance*	*With impact on ecosystem functioning or polluting the surrounding environment*
• Foliar N > 2% • Cuticular wax erosion • Foliar injury • Loss of apical dominance • Drop of foliage	• Exceeding the compensation point • Compensation point is elevated due to NH_3 exposure • Increased glutamine and arginine content, • elevated GS and NR activity • Immobilisation on N in litter • chlorosis	• Foliar injury • Increased winter desiccation • Loss of apical dominance • Drop of foliage • chlorosis	• Increased SLA, S/R, .. • Altered biomass production • Imbalanced nutrient status • Impact on photosynthesis • Increased winter desiccation • Change in reproduction • reduction in stress tolerance • Exhausting soil buffer capacity • increased nitrate and ammonium concentration in edaphic ground water	• Building up tolerance of vegetation • Altered biomass production • Increased winter desiccation • Reduced lifespan (esp. of trees and heath) • (drop of foliage) • Change in reproduction • Change in stress tolerance • Leaching of minerals • Evaporation of N compounds • Exhausting soil buffer capacity

3. Materials and methods: relevance of lab experiments and evaluation techniques

Laboratory fumigations with NH_3 have resulted in quite some information on working mechanisms on uptake, physiology and toxicity. To assume similar concentration levels in laboratory fumigations and in the field to have similar effects is a risky assumption. Certainly, climatic conditions, especially the temperature profile, are strongly determining the detoxification capacity of plants for NH_3 (probably more than with O_3, SO_2 etc). And climate conditions in the field are not easy to simulate on laboratory scale.

In this respect, field studies are better, including field fumigations, transects studies and field surveys. But local conditions, local history and the occurrence of rare events may make the results of field studies difficult to reproduce or to generalise, especially as long as there are only a handful of those studies. A trap in field manipulation experiments can be that the control treatment in not “clean”. The observation of lower sensitivity of Dutch & German heath land compared to those of Scotland and Scandinavia may also reflect the fact that, with lower background N deposition and NH_3 concentrations in Scotland and Scandinavia, it becomes possible to detect effects at lower levels.

Ecosystem simulation models could be useful, but are still in a preliminary state of development for this purpose.

This dilemma is not unique for air pollution research. In soil and water pollution the problem has been "solved" by either using a "safety factor". In fact, there are good reasons, both from the scientific and the political point of view, to use similar evaluation techniques and safety levels when evaluating risks of air, water or soil pollution. But current practice is different. Standards for soil and water pollution are based on short term tests with algae, daphnia etc. with lethality as effect criterion, while standards for air pollution are based on long term tests with higher plants and first sign of injury as criterion.

In toxicology the term for "safety factor" is "assessment factor". It aims to cover the uncertainty caused by lack of knowledge on inter- and intra-species variability, on extrapolating from short to long term exposure and from lab to field. An assessment factor of 10 is most common for ecosystem protection, but in some cases there are reasons, both scientifically and politically, to choose for higher and lower assessment factors.

The CLE of 8 $\mu g\ m^{-3}$ for an annual average (van der Eerden *et al* 1991) was based on a statistical evaluation technique that is relatively often used in soil and water pollution. No assessment factor was used. The scientific basis of this 8 $\mu g\ m^{-3}$ may have been the best there was at the year of evaluation, but it has many draw backs.

In the case of NH_3, the currently available database on effects contains information on inter-species variability, on short and long term exposure and on data from both laboratory and field experiments (Annex 1). Although these different types of information are not yet well structured and may be biased, one could ague whether a safety factor of 10 is really needed. Apart from these thoughts, one has to be aware that a safety factor proposed by environmental scientists is subject of debate when it comes to application in practice. Availability of technology, societal pressure, economical consequences, political agenda and other items are determining application.

A technical (and financial) problem with field studies is also that continuous NH_3 monitors are expensive. This is why generally damage is related to average long-term concentration, which are easily measurable e.g. using reliable passive sampling, while evidence exists that peak concentrations can be crucial in developing effects. This points to the need to resolve the interface between mechanistic understanding and practical approaches that can be used for policy monitoring. For example, large peak concentrations correspondingly increase the monthly and annual mean concentrations. Hence, based on the characteristic geometric standard deviation of concentrations, monitoring on a monthly basis may be sufficient for a practical assessment of the environmental effects of gaseous NH_3.

4. More considerations on exposure / response

4.1. Temporal aspects of exposure: short term fluctuations and exposure history.

Generally NH_3 is emitted by local sources at a height of 5 metres or lower. The effective emission height from stables may even be at surface level due to eddies and turbulent "downwash" of air downwind of the stable, in addition to emissions from grazed and manured land. This is why near to sources the ratio between peak and mean concentration is relatively high. The more isolated the source and the lower the regional background concentration, the higher this ratio is.

A relevant question in the scope of CLE setting is if it matters whether an average exposure level consists of high peaks combined with low levels between the peaks, or of a more constantly moderately elevated level. The relevance of answering this question is manifold:

- it indicates how results of fumigation experiments with constant exposure levels should be used at deriving CLEs,
- it indicates effectiveness of emission abatement strategies. *e.g.*
    - a chimney reduces local peaks but increases regional background levels.
    - to distribute emission over more sources has the same effect, pointing to the need to set limits to emission quantities,
    - to limit emissions during sensitive periods of the year and allow higher emissions during other periods reduces critical events, but the annual deposition remains unchanged.

In regard to the interpretation of observed results, the importance of concentration variations may mean that effects occur at a lower long-term mean NH_3 concentrations in the field (where concentrations fluctuate substantially) than in OTC studies (where NH_3 concentrations may be regulated to a relatively stable value).

Regarding short-term exposure, a level exists above which cellular membranes and plant tissues are destructed. This level is for most plant species probably (considerably) higher then 100 µg m^{-3} for one day and more then 1000 µg m^{-3} for one hour (van der Eerden et al. 1991). With lower exposure levels, the impact is determined by the rates of uptake and release as related to the detoxification capacity. The uptake velocity is determined by stomatal conductivity and the ratio between internal and external NH_3 concentration. The level at which an equilibrium exists between internal and external concentration is the compensation point, as already noted.

Based on these features one might assume peak exposures were at least as important as the mean exposure level. Exposure to a peak concentration results in uptake until the compensation point is reached and in release when the peak exposure is followed by a considerably lower exposure level. The concentration level between two peaks thus may not be relevant if the next peak follows soon. The graph below (Figure 1) illustrates this reasoning.

This phenomenon was clearly proved with accumulation of atmospheric fluorides in grass (Van der Eerden 1991). To our knowledge there is no current data with which to extend something similar for NH_3, and this remains a priority for future research. One difference is, of course, that under favourable climate conditions NH_3 can be easily and quickly metabolised. In those conditions the period between two peaks where the NH_3 concentration is irrelevant is shorter.

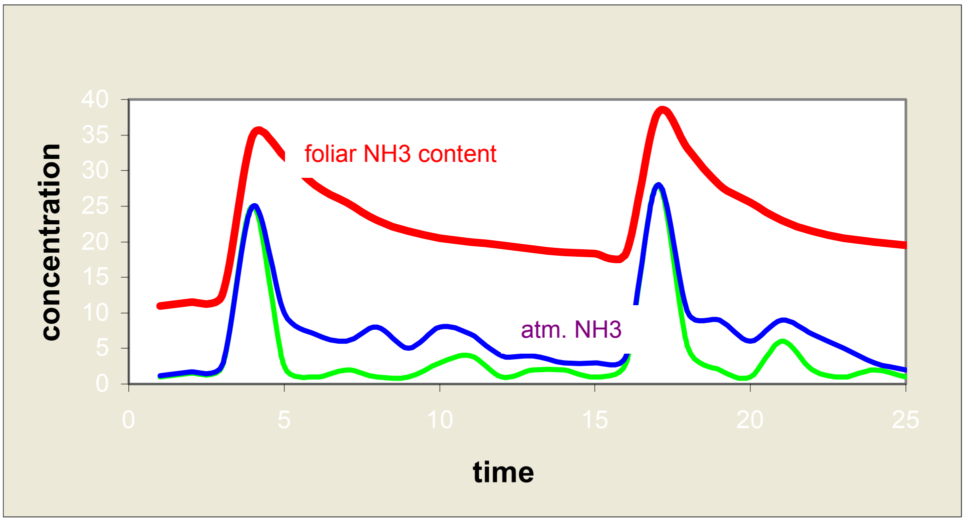

Figure 1: Illustration of the possible relationships between NH_3 exposure in time (blue line), cellular/apoplastic ammonium concentration (green line) and an integrating parameter such as foliar nitrogen (red line)

The reasoning discussed above gives evidence to state that one has to be careful with using the results of experiments with constant exposure levels for derivation of CLEs: they are probably underestimating the effects in the field. The compensation point for uptake of atmospheric NH_3 is much smaller for natural vegetation than for agricultural vegetation. Hence, the same phenomenon could take place at a lower concentration level.

Annex 2 of this paper presents two evaluations of the concentration pattern around local sources. One indicates that CLEs for short term exposures are exceeded more frequently than CLEs for long term exposures. Another study concludes the opposite. The difference will relate to the geometric standard deviation of the NH_3 concentration at different sites.

Very relevant in the scope of temporal distribution is, of course, the long term concentration pattern. In the previous section we mentioned already the gradual loading of the litter layer and changing sensitivity of vegetation: both indications exist for adaptation as well as for increase in sensitivity.

Another temporal aspect is that we may have to wait for adverse conditions to see effects. Field observations suggest that exceeding the CLE not at all guarantees development of injury. To cause injury, it is possible that a slight exceedance must be accompanied with adverse conditions like low light intensity, an early night frost, drought stress, a storm (having more impact to trees with too big crowns), an infestation by a plague or disease etcetera (van der Eerden *et al* (1991). This is in coherence with the observations by Sheppard et al. (2006, personal communication) who observed damage to *Cladonia portentosa, Sphagnum capillifolium* and *Calluna vulgaris* all through apparently different mechanisms but often linked to a stress interaction with NH_3, such as dessication.

It must also be recognized that ambient NH_3 concentrations vary substantially in time. Even if the mean concentration is not substantially higher than a long term CLE, a short term peak concentration may combine with adverse conditions. Such combination events happen infrequently, so that actual damage may only be triggered after several years.

To our knowledge, only one study exists dealing with the frequency of occurrence of toxic combinations of high NH_3 concentrations and adverse conditions (Sheppard et al. 2006).

4.2. Spatial differences in uptake and sensitivity.

With similar levels of exposure to NH_3 the response of vegetation may differ in space, both on the local scale and EU wide. Reasons could be:

- difference in wind velocity,
- presence of other pollutants, enhancing the deposition velocity or acting synergistically. Especially SO_2 is probably relevant in these respects,
- different ratios of peak to mean concentration, duration of high concentrations (see previous paragraph),
- different uptake rate due to different temperature (the compensation point doubles every 5^{o}C),
- different plant sensitivity due to climate, soil quality or plant morphology,
- difference in degree of adaptation to NH_3.

A relevant issue for this workshop is whether these differences call for different CLEs in different climate zones of Europe, or that these differences are negligible compared to the uncertainties elsewhere in the derivation of CLEs.

5. Evaluation

If virtually every measurable response to NH_3 should be considered to be an adverse effect, then probably a treasure of unpublished information exists. If, on the other hand, only effects of definite ecological relevance should be used, then much of the basis of a CLE for NH_3 cannot be considered. For the short term there is no other choice than to let a team of experts judge which response data can be used. This workshop is a unique opportunity for this judgement. Table 1 could be useful in this exercise.

There are good, mainly practical reasons for different approaches in air, water and soil pollution. But at a moment like this, where critical levels are re-assessed, one should carefully consider options to bring more similarity in risk assessments.
Given a set of relevant effective responses a good choice is still to use the statistical techniques that were already used by van der Eerden *et al.* (1991), although several improvements are at hand. See Annex 1 for more information. On the longer term, a connection with causal analytical models has to be found. This brings CLEs in relation with mechanistic models for atmospheric dispersion, forestry production and soil chemistry.
Key new information is also available from studies of actual changes under field conditions. CLE estimates derived from such datasets provide an independent assessment to the toxicological model estimates.

Validation of CLEs in practice is a challenge, though not easy. With good reasons, politicians and the lay public demand explanations on apparent contradictions that they recognise.
A useful exercise in this respect is to explain apparent controversies in the results of heathland research in different countries: see previous section in the paragraph on "adaptation".
A challenge is also to explain the absence in many cases of certain effects in the direct neighbourhood of local NH_3 point sources: see Annex 2.

In considering the overview of knowledge, one can conclude that much information is available on NH_3 effects and on influencing factors, but also that much of this information of a qualitative nature. In this stage it seems appropriate to continue re-assessing a CLE along two lines:

- a basic approach in which only proven ecophysiological effects are taken into account and in which NH_3 is assumed to be the only pollutant. In this approach physical (e.g. exceeding the level of the compensation point), chemical (e.g. increased N content) and biochemical effects (e.g. increased arginine content) would be neglected. Additive or interactive effects with other pollutants are neglected as well. With this approach the highest non-effective concentration is defined. Whether a safety factor is needed or depends on the quality and coverage of the information left with this selection. The height of such a safety factor could be derived from comparison with risk assessments on other environmental problems. In this respect it is worth to note that in the Critical Loads, derivation uncertainty due to a poor database is not translated into a safety factor, but in a remark that such a critical load is just a "best estimate".
- a complete approach in which in which all types of effects with a causal relation to ecological endpoint are included and where interactive effects, including those caused by other pollutants, are considered. In this approach causal-analytical eco-system models should be used for generalisation. And a statistical analysis should provide confidence limits.

The complete approach will end up with a more reliable, probably lower critical level compared to that derived with the basic approach. On the other hand, the boundaries between these two approaches need to be further debated. Similarly, a distinction is necessary between the immediate need to revise NH_3 critical levels based on new data and the longer term research challenge to refine the approaches in defining NH_3 critical levels. The ammonia workshop requires that both these needs are addressed.

Annex 1

History and future of the CLE for NH3 of 8 µg m^{-3}.

In 1991 Van der Eerden *et al* published CLEs for NH_3: 8, 23, 270 and 3300 µg m^{-3} for means over a year, month, day and hour, respectively.

The majority of data were from the early eighties or older (*e.g.* Van der Eerden 1982). Fumigation experiments in those times had their limitations. Concentration control was done with equipment that had a detection limit around 10 µg m^{-3}, the control treatment was generally not completely free of NH_3 and climatic conditions were not well reproducible. More-over, the choice of plant species was generally based on goals like finding sensitive species that could be used as indicators in the field, or producing background in formation for the evaluation of damage claims. This is why in many experiments tomato, cabbage and conifer saplings were used.

In the late 1980s the goals of NH_3 fumigations shifted towards concern on natural vegetation. Heathland species and bryophytes were added to the selection of species considered. The concentration control had become better, but was still poor for levels below 10 µg m^{-3}.
In the 1990s, the financial support for NH_3 fumigations largely ceased and few fumigations have been done since then.

The WHO published guidelines for the first time in 1987. In 1997 a re-evaluation was executed by IPCS and in 1999 by the WHO. In these re-evaluations the following table was shown (some details where added by Van der Eerden in 2006 for more clarification).

Table A1.1: Three lowest exposure levels (in µg m^{-3}) per exposure length and endpoint, at which NH_3 caused significant effects. (After WHO 1997). If less than three levels are mentioned in one cell, this means that no relevant information was available.

	(bio-)chemical	Physiological	growth aspects
long term	50; 8 months [1]	53; 9 months [7]	25; 1 year [10] 53; 8 months [11] 35; 16 months [12]
growing season or winter	100; 6 weeks [2] 60; 14 weeks [3] 180; 13 weeks [4]	50; 6 weeks [8]	60; 2 months [13] 20; 90 days [14] 30; 23 days [15]
air poll. episodes	2000; 24 hrs [5] 213; 5 days [6]	213; 5 days [9]	120; 11 days [16] 1000; 2 weeks [17] 300; 3 days [18]
short term			30,000; 1 hr [19] 2,000 2 hrs [20] 2,000 6 hrs [21]

[1] Species of *Violion caninea* alliance; imbalanced nutrient status (Dueck & Elderson, 1992)
[2] *Deschampsia flexuosa;* change in amino acid composition (Van der Eerden *et al.* 1990a)
[3] *Pinus sylvestris*; increased GS activity in Pine (Pérez-Soba *et al.* 1990)
[4] *Pseudotsuga menziesii;* imbalanced nutrient status (Van der Eerden *et al.* 1992)

[5] *Lycopersicum esculentum*; increase of NH_4^+ in the dark (Van der Eerden 1982)
[6] *Lolium perenne*; 30 % of N in the plant is derived from foliar uptake (Wollenheber & Raven 1993)
[7] *Pinus sylvestris*; increased loss of water after two weeks of desiccation (Dueck *et al.* 1990)
[8] *Populus sp.*; increase in stomatal conductance in leaves; increase in mesophyll conductance and maximum photosynthetic rate at a slightly higher exposure level (Van Hove *et al.* 1989a)
[9] *Lolium perenne*; significant impact acid/base regulation and nutrients status
[10] *Pseudotsuga menziesii;* erosion of wax layer (Thijse & Baas, 1990; the authors have some doubts about the causality of this effect (pers. comm.)
[11] *Calluna vulgaris*; reduction in survival rate after winter (Dueck 1990)
[12] *Arnica montana*; reduction survival after winter and of flowering (Van der Eerden et al 1991)
[13] field exposure during winter; median concentration; severe injury of several conifer species (Van der Eerden 1982)
[14] This figure is an estimate based on a fumigation experiment with five heathland species. Regression analysis was used to estimate the concentration at which 50% stimulation of shoot growth was reached (root growth was not significantly or much less stimulated).
[15] *Racomitrium lanuginosum*; chlorosis (Van der Eerden *et al.* 1991)
[16] *Hypnum jutlandicum*; chlorosis (Van der Eerden *et al.* 1991)
[17] *Lepidium sativum*; reduction in dry weight (Van Haut *et al.* 1979)
[18] several horticultural crops; leaf injury (several authors, see Van der Eerden 1982 for details)
[19] various deciduous trees; leaf injury (Ewert 1979)
[20] *Brassica sp, Helianthus sp*; leaf injury (Bennedict & Breen 1955)
[21] *Rosa sp.*; leaf injury rose (Garber 19**35** (!))

In air pollution research it is common to base CLEs on the NOEC of the most sensitive species and the most responding effect that are assumed to be relevant. In practice, the NOEC is assumed to be slightly below the lowest effective exposure level (depending on the expert interpretation). With this approach the 270 and 3300 µg m^{-3} for means over a day and an hour are based on the sensitivity for foliar injury of various crops including tomato, buckwheat, sunflower, wheat, some deciduous trees and rose.

The CLE of 23 µg m^{-3} for monthly mean is largely based on sensitive bryophytes on two experiments:

- one species (*Racomitrium lanuginosum*) out of four showed leaf injury after at 30 µg m^{-3} after 23 days. Regression analysis suggested that a NOEC probably is well below 30 µg m^{-3}.
- Five heathland species were fumigated for 90 days with 6 concentration levels. Regression analysis was used to estimate the concentration at which 50% stimulation of shoot growth was reached (root growth was not significantly or much less stimulated). *Viola canina*: 21 µg m^{-3}; Variance accounted for NH_3: 66%, *Agrostis capillaries*: 13 µg m^{-3}; Var 54%, *Antenaria dioica*: 49 µg m^{-3}; Var 59%, *Calluna vulgaris*: 41 µg m^{-3}: Var 67%, *Potentilla erecta*: 145 µg m^{-3}: Var 49%.

With the approach of assuming the CLE to be equal to the NOEC of the most sensitive species, the 8 µg m^{-3} for an annual mean were the following:

- In a 16 months open-top-chamber fumigation several effects on heath land species were found, although some of them could only be indicated by regression analysis. The experiment had two controls: one charcoal-filtered and one unfiltered, having a NH_3 concentration of 3 and 6 µg m^{-3}, respectively. The lowest out of four fumigation levels was 35 µg m^{-3}. Nearly all species responded on 35 µg m^{-3} compared with the two controls. Increasing shot/root ratio, reduced flowering and reduced survival after winter were most common. Indications were found for a NH_3 effect in the 6 compared with 3 µg m^{-3} on *Arnica Montana* (see van der Eerden et al. 1991, Figure 5). But these were difficult to interpret, because the difference between filtered and non filtered air was not only a difference in NH_3 concentration.

- *Calluna vulgaris* was fumigated for 12 months with 4 concentration levels. The control treatment had a NH_3 concentration of 4 µg m^{-3} and lowest fumigation level was 25 µg m^{-3}. 3d instar larvae of the heather beetle (*Lochmea saturalis*) was fed for 7 days with fumigated Calluna shoots. It grew significantly faster with higher NH_3 concentrations. Regression analysis suggested that a NOEC probably is well below 25 µg m^{-3}.
- A consideration was also that the CLE for an annual mean can not be higher than the CLE for a monthly mean.

The approach of assuming the CLE to be equal to the NOEC of the most sensitive species, and the necessity to include expert judgement is not very satisfying. More-over, the notion that only the most sensitive species is taken into account, while the data base contains information on species variation in sensitivity is not satisfying as well.

Therefore, Van der Eerden *et al* (1991) decided to use a statistical evaluation technique, that is relatively often used in soil and water pollution. It is based on an estimate of the level at with 95% of the species are protected with a probability level of 95%. The assumption was that the inter species variability in sensitivity has a log-normal distribution. The method allows other protection levels and other probabilities, but these were used in 1991 for the CLE of NH_3. Figure 1 indicates the methodology. It was developed by Kooijman (1987) and later shaped and improved by Aldenberg & Slob (1993) and others. In the scope of standard setting it is currently widely in use, also in EU environmental policy, although criticism exists as well.

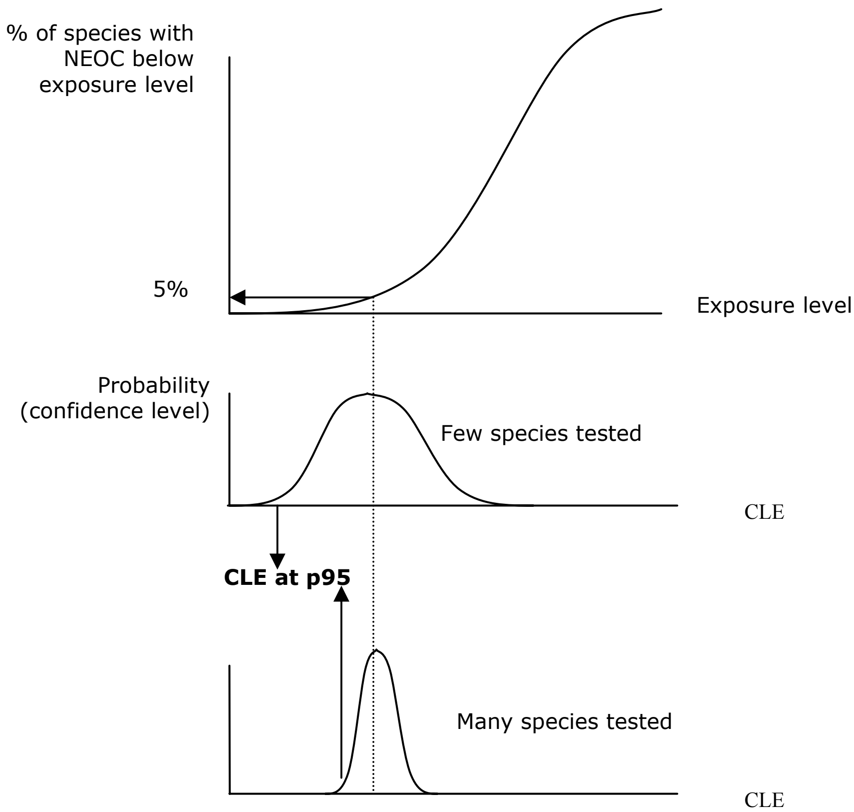

Figure A1-1. Indication of a CLE derived from a frequency distribution of sensitivities.
The upper graph shows this distribution. The estimated level at which 95% of the species is protected is indicated. The two lower graphs show the level of confidence of this estimate. The more species tested the more reliable the estimate of the CLE is (and the nearer the CLE at P95% approaches the P50of the real CLE).

Evaluation

The 8 µg m^{-3} for an annual mean is an estimate of a CLE for 95% of the species a probability level of 95% (CLE prot95_prob95). No assessment factor was used (see Section 2).
CLE prot90_prob95 and CLE prot95_prob50 were much higher. The assumption of log-normality of the inter species variability was not very influential. A log-logistic distribution resulted in similar CLEs.

The scientific basis of this 8 µg m^{-3} may have been the best there was at the year of evaluation: 1991, but it has many draw backs:

1. A variety of responses have been included: toxicity, growth simulation, increased shoot/root ratio etc. Its direct relevance for protection of the species or ecological risks on ecosystem level is unknown or doubtful in many cases.
2. The climate conditions and the concentration patterns in the lab experiments did not simulate any field situation.
3. The concentration levels used were generally a manifold of those in the field situations.
4. The representativeness of many of the tested plant species for threatened species or vegetations is unknown or doubtful.
5. The statistical evaluation could be criticised as long as it is not clear which frequency distribution (log normal; normal etc) of species sensitivity is most realistic.

One could think about using the same evaluation method, but improving the input.
New literature information confirms that NH_3 effects happen at in the concentration range of 1-25 µg m^{-3}. Kupcinskiene (2001) showed significant erosion of the cuticular wax layer of *Pinus sylvestris* needles at concentrations below 23 µg m^{-3} and regression analysis on field trials on acid moorland species by Leith *et al* (2001 and 2002) suggests effects below 20 µg m^{-3} as well.

Recent information produced by Frati et al., (2006) and Wolseley et al. (2006) shows obvious responses of epiphytic lichen and sphagnum species in the range of 1-2 µg m^{-3} (with an expert judgement of an uncertainty range of 0.6-3 µg m^{-3}). Results will be presented in this workshop.

A trap at replacing information that is out of date by this new information is that the narrow scope of the past: too many crops is replaced by a selection that is strongly dominated by lichens. In an EU risk assessment workshop experts proposed, as a general rule, a minimum of 15 species within a minimum of 3 taxonomic groups. This is probably achievable with the available NH_3 data.

The draw backs mentioned under bullets 1, 2 and 3 apply to most of the databases for environmental standards. In that respect, the CLE for NH_3 is not an exception.
In most toxicological studies, the uncertainty created by bullets 1-4 are covered with an assessment factor (generally of factor 10). .

Concerning bullet 4, to include more species from natural vegetation (and to exclude at least some of the crops) certainly improves the data base. Such a change can also be motivated by Pearson & Stewart (1993), who showed that species of a climax vegetation on N-poor soils are always relatively sensitive for NO_x and NH_y. On the other hand, to select only species that are assumed to be sensitive reduces the information on variation in sensitivity (see bullet 5). The listing of reported NH_3 effects contains several species. Some species or taxonomic groups may be over represented and some species, *e.g. Calluna vulgaris*, are represented with several endpoints. One option is to use only the most sensitive end point, but in that case the observation that a species can respond differently in different experimental settings (and in different EU regions) is neglected.

Concerning bullet 5, one could consider to use the frequency distribution of N preferences, as indicated by Ellenberg-N, of species that are generally assumed to be crucial for natural ecosystems (Ellenberg 1991).

Of course, a causal-analytical model that simulates ecosystem responses is to be preferred over statistical approaches. Existing mechanistic models for the performance of forests, heath land and crops have an entry for N. The response of those systems to NH_3 can be simulated if uptake and detoxification capacity can be quantified. Although first attempts (Van der Eerden *et al.* 1998) were not very promising for several reasons, further research is certainly useful and may result in information that can be used in the next update of guidelines.

Evaluation in the scope of this workshop

Since 1991 there have been few studies of laboratory NH_3 fumigations, but there have substantial new datasets from field studies, particularly transects away from point sources and regional scale assessments. A key feature of many of the field studies is that it is often possible to address the long-term (multi-year) effects of NH_3 that it was not possible to address in the former estimates of critical levels. In the field, effects of NH_3 appear in many cases to be detectable at NH_3 concentrations lower than the annual CLE of 8 $\mu g\ m^{-3}$. Such differences are not surprising and reflect:

- The need to set a CLE for long term protection to NH_3 concentrations (over many years), which is expected to be smaller than the annual CLE.
- Ammonia measurement methods have improved and many of the field studies compare exposures against background concentrations which are much cleaner than in the former fumigation experiments. This allows effects to be detected at lower levels.
- More attention has been given to sensitive plant groups, such as epiphytic lichens in sensitive contexts (e.g. acidophyte lichens growing on twigs)
- It is possible that exposure to naturally fluctuating NH_3 concentrations in the field leads to more adverse effects than exposure to little fluctuating NH_3 concentrations in fumigations studies for the same long-term mean concentration (although this remains to be demonstrated).

As a result, particular attention is needed in this workshop to compare the results the available field and laboratory studies and to consider a CLE for long term protection of specified habitats.

Annex 2

How to judge the (absence of) local effects of atmospheric ammonia?

On distances less then 50 m from big ammonia sources, where, based on assumed deposition velocities, the nitrogen deposition should be lethal for most plant species, the effects are often detectible, sometimes obvious, but rarely disastrous for many plant species. This supports the suggestion made by several scientists, that with high exposure levels plants may build up a resistance to uptake or have the possibility to re-emit part of the uptake.
A related explanation is that the deposition velocity reduces at very large NH_3 concentrations (e.g. in the range 8-100 $\mu g\ m^{-3}$), due to tendency to saturate cuticular uptake processes (Sutton et al. 1993, Flechard et al. 1999, Jones et al. unpublished data. Although often visible injury at the vegetation in this 50 m zone may not be seen, such high NH_3 concentrations have been shown to have major effects on the community composition of semi-natural habitats (see below).

Evidence for local effects

Effects on forests, natural vegetation, arboriculture and crops in the direct neighbourhood of ammonia sources are evaluated among others in Kúhne (1966), Garber & Schürmann (1971), Hunger (1978), Tesche & Schmidtchen (1978), Ewert (1978), Rudoph (1981), Kaupenjohann *et al* (1989), Hoffmann *et al* (1990), Pitcairn *et al* (1990), Fangmeijer *et al* (1994), Pitcairn *et al* (1998), Pitcairn (2002), Frati et al., (2006) and Wolseley et al. (2006). Probably a lot more information on local effects is available although not easily assessable in non-scientific literature, like damage claims and periodical assessments by forestry managers[5].

Notwithstanding the body of evidence for local effects, one must be aware of the numerous observations, generally not recorded in scientific literature, where species appear to survive even though critical levels are substantially exceeded. One of the possible reasons for this phenomenon may be that effects are only caused by a co-occurrence of peak concentrations with unfavourable physiological conditions.

Relevance of peak concentrations

Plants can build up resistance to uptake by elevating its compensation point, or increasing its tolerance by increasing detoxification capacity. Nevertheless, if phytotoxic effects occur, it is probably due to peak concentrations. For example, actual observations of damage tend to follow periods with peak NH_3 concentrations. Conversely, the contributing role of increased mean concentrations cannot be ruled out, as illustrated by reducing thresholds for effects following exposure longer than one year (Sheppard et al. 2006), as well as the interaction of NH_3 with substrate pH (e.g. Wolseley et al. 2005).

[5] A probably not yet fully explored source of valuable information is the data bases from Eastern European countries at the communistic era. Publications like that of Hoffmann *et al* 1990 suggests that useful info may be discovered.
Several of the mega-farms that existed in Eastern Europe up to some 15 years ago caused severe damage to the surrounding vegetation (Hoffmann, Pers. Comm.). Most of them have put out of operation. It might be that measurements and observations have been done that describe the recovery process. If so, this is highly valuable information in the scope of risk assessment.

Van der Eerden *et al* (1998) evaluated the probability of exceeding CLEs in the direct neighbourhood of NH_3 sources. They used the CLEs suggested by Van der Eerden *et al* (1991): 8, 23, 270 and 3300 µg m^{-3} for a year, month, day and hourly average NH_3 concentration. They derived the duration of higher concentration levels from field measurements (about 100.000 hourly means) and concluded that CLEs are especially exceeded for 1 to 10 days exposures, so not for shorter or longer term exposures.

By contrast, Burkhard et al. (1998), examined the maximum hourly, daily, monthly and annual NH_3 concentrations from long term continuous monitoring in Scotland and compared these to the respective critical levels for these periods. They found that none of the CLEs were exceeded, but that the gap between observed concentrations and the CLE was smallest for the annual period, and increasingly larger for shorter periods. They concluded that, for sites with a characteristic geometric standard deviation of mixed agricultural landscapes, the CLE for shorter periods would only be exceeded where the annual CLE was already exceeded (since the peaks contribute to a larger long term mean).

The difference between these two examples may reflect the uncertainty on the relative importance of manure spreading and other manure handling causing high concentratios for the short term. Where more or less continuous point sources dominate, it is likely that the annual CLE will be exceeded first. However, it is feasible that occasional manure spreading events could lead to exceedance of the daily/monthly critical level, while not exceeding the annual CLE.

Critical distances in science and in practice:

Van der Eerden *et al.* (1998) designed a decision support system that relates NH_3 emission of a farmhouse to the distance until where CLEs are exceeded. As an option the regional background concentration, the exposure caused by NH_3 sources in the direct neighbourhood and roughness of the surface between the source and the exposed object could be accounted for. For instance, with a source of 1000 kg yr^{-1} a critical distance was calculated to be around 150 m. The approach is simple, elegant and scientifically sound. But once it was used in legislation in the Netherlands, and in juridical procedures on objections against building permits, the judge found it too new, too complicated and too much based on a momentary local situation. He decided to continue using the *juris prudence*, made more than 30 years ago, that distances between critical object and source (independent of its emission, background concentration etc) of less than 50 m for conifers and nature, and 25 m for other horticultural crops should be avoided.

This decision that was very mild towards animal husbandry, but did not result, up to now, in significant cases of visible injury. Of course, this observation does not imply that no adverse effects occur at more than 50 m from the source. Sensitive species could have been wiped out and tolerant species are dominating, for instance. But it stresses the need for information on variation in sensitivity to NH_3, and may be for not only assessing one CLE that accurately protects the most sensitive species, but also one CLE with a lower protection level.

Annex 3

Relation between critical levels and critical loads for N-compounds.

Both Critical Levels (CLE) and Critical Loads (CLO) are intended to be set so as to protect vegetation. Multiplying a CLE with deposition velocity results in a CLO. Thus, it might seem superfluous to set both CLEs and CLOs. Current practice is different, however (Table A3.1). This is at least partly due to difference in scientific source: assessing CLEs was generally triggered by air quality specialists, while CLOs generally have their basis in ecology. Currently, CLEs are especially useful in emission abatement, while CLOs are useful in nature conservation. There seems to grow a preference in NH_3 policy and at rural planning issues to directly use NH_3 concentrations, thereby avoiding the uncertainty in calculation deposition at the site level.

While this represents current practice, there is significant potential for better harmonization between the two approaches.

Table A3.1. Current differences in practice between Critical levels (CLE) and Critical Loads (CLO) for N-containing air pollutants. There is a need for better harmonization between the two approaches.

	CLE	*CLO*
Summarized definition	Concentration above which effects do occur	Deposition below which effects do not occur
Exposure duration:	short term (1 yr or less)	Long term (+ 10 yrs)
Effect of peak exposures	Included	Neglected
Agent:	Separate CLE for each N-compound	All N-compounds added
Object of interest:	Individual plant or crop	Natural vegetation or forest plantations
No effect concentration:	Generally: the lowest significant response in experiments (*e.g.* 10% yield reduction)	Generally: a “safe” concentration derived by extrapolation or modelling.
Goal:	Protection of sensitive plans species	Protecting proper functioning of ecosystems
Combination effects	Possibility of synergism is considered	Additively is presumed

It should be a goal of future research to make accurate conversion possible of CLE and CLO into each other, and so to make more comparable for their complementary roles. Several bridges have to be build to achieve this goal. Major gaps in knowledge in this respect are:

- much about the deposition behaviour of N-compounds, especially of NH_3 is poorly quantified (*e.g.* cuticular saturation at very large NH_3 concentrations and subsequent re-emission potential), introducing uncertainty to the CLO.
- CLEs for exposure periods of more than one year need to be estimated
- CLOs for different N compounds are need to be better estimated
- CLEs should include more relevant ecological information
- Both CLEs and CLOs using empirical approaches can not fully differentiate between different climate zones.

Annex 4

The relevance of distinguishing different N compounds

Part of the relevance of distinguishing different N compounds is illustrated in Figure A4.1. It shows an indication of the impact of three gaseous N-compounds on photosynthesis being very different if expressed in terms of deposition. See WHO(1997) for background information.

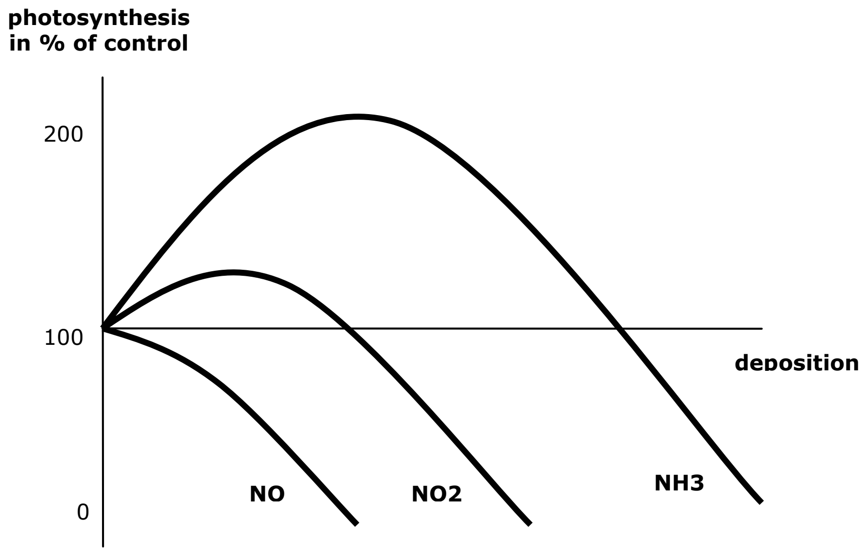

Figure A4.1. Indication of exposure/ response relationships for the impact of N compounds on photosynthesis.

The fertilizing effects of NH_3 and NH_4 at low and medium high concentration levels are obvious and well documented. Interesting is the observation made by Leith *et al* (2001 and 2002) that with the same N input growth stimulation by NH_3 is stronger than that of NH_4^+. NO only makes a trivial contribution to N deposition (it is actually emitted by soils of high nitrogen status), but can not be neglected because of its toxicity.

With NO_2 fertilising effects are possible, but toxicity dominates. Ashenden *et al* (1993) found no obvious relation between sensitivity to NO_x and the nitrogen preference as indicated by Ellenberg (1985).

Other reasons to differentiate are:

- Some N-compounds that have a minor contribution to N deposition are relevant for other reasons:
    - NO and NO_2 are precursors for tropospheric O_3, which acts as phytotoxin and greenhouse gas. NO is mainly emitted by urban sources, although soil emission should not be neglected.
    - N_2O is emitted by soil as a result of denitrifying the excess N in the soil. It adds to depletion of stratospheric O_3 and thus to increasing UV radiation.
- Differentiation makes it possible to take combination effects into account. For instance:
    - a strong synergism exists between SO_2 and NO_2. Interactions between other compounds (SO_2, NH_3, O_3 etc) are more variable
    - NO_x inhibits the fertilising effect of CO_2. With other N-compounds this is not know

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