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Combination of toxicological and epidemiological approaches for estimating the health impact of atmospheric pollutants. A proof of concept for NO_2

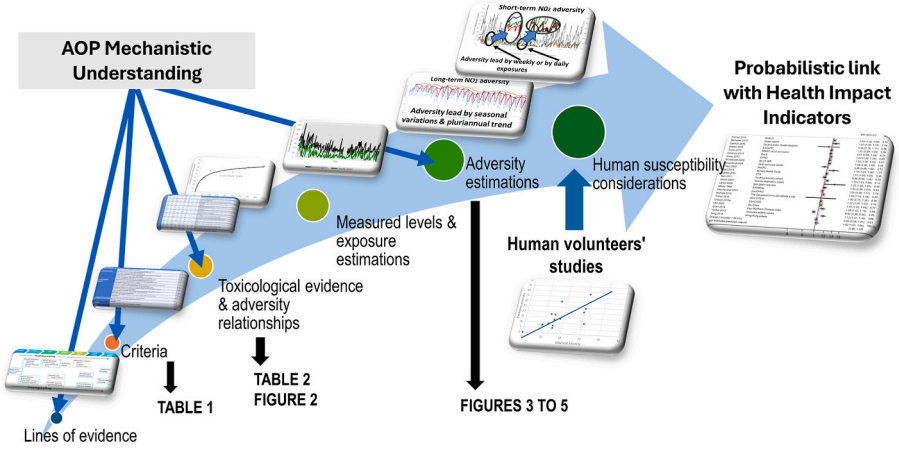
Susana Pallarés Porcar [a,1], Francisco Javier Sánchez-Íñigo [a,1], Beatriz Nuñez-Corcuera [a], Joaquín Lozano Suárez [a], Sonia Arca-Lafuente [b], Clara Moyano Cárdaba [a], Ana Fernandez Agudo [c], Mercedes de Alba-Gonzalez [c], Rebeca Ramis [d,e], David Galán-Madruga [a], Maria del Carmen González-Caballero [c,*], Verónica Briz [b], Susana Guevara-Hernandez [a], Ma Encarnación de Vega Pastor [f], Denis Sarigiannis [g,h,i], Saul Garcia Dos Santos [a], Jose V. Tarazona [c]

[a] Department of Atmospheric Pollution, National Environmental Health Center (CNSA), Instituto de Salud Carlos III (ISCIII), Madrid. Spain
[b] Viral Hepatitis Reference and Research Laboratory, National Center of Microbiology, Institute of Health Carlos III (ISCIII), Madrid, Spain
[c] Risk Assessment Unit. National Environmental Health Center (CNSA), Instituto de Salud Carlos III (ISCIII), Madrid, Spain
[d] Department of Epidemiology of Chronic Diseases, National Center for Epidemiology, Instituto de Salud Carlos III (ISCIII), Madrid, Spain
[e] Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain
[f] Department of Air Quality. Urban Planning, Environment and Mobility Area, Madrid City Council, Spain
[g] Aristotle University of Thessaloniki, Department of Chemical Engineering, Environmental Engineering Laboratory, University Campus, Thessaloniki, 54124, Greece
[h] HERACLES Research Center on the Exposome and Health, Center for Interdisciplinary Research and Innovation, Balkan Center, Bldg. B, 10th km, Thessaloniki-Thermi, Greece
[i] University School of Advanced Study IUSS, Piazza della Vittoria 15, 27100, Pavia, Italy

HIGHLIGHTS

- Extending AOP as Health Impact Pathways-HIP gives quantitative adversity estimates.
- The HIP conceptual approach integrates experimental tox and epidemiological data.
- Nonlinearity of NO_2-related long/short-term health impacts must feed trend analysis.
- The estimates confirm the need to independently assess different population groups.

GRAPHICAL ABSTRACT



* Corresponding author. Risk Assessment Unit, National Centre for Environmental Health (CNSA). Instituto de Salud Carlos III (ISCIII). Ctra. Majadahonda a Pozuelo, Madrid. 28222, Spain.
E-mail address: mcgonzalez@isciii.es (M.C. González-Caballero).
[1] Shared first-author.

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ABSTRACT

Background: Regular monitoring of the air pollutant nitrogen dioxide (NO_2), an indicator for traffic-related emissions, is a priority in urban environments. The health impacts associated with NO_2 exposure are the result of a combination of factors, including concentration, duration of exposure, and interactions with other pollutants. WHO has established air quality guidelines based on epidemiological studies.
Objective: This study develops a new concept "Health Impact Pathways (HIPs)" using adversity as a probabilistic indicator of health effects. For this purpose, it integrates available toxicological and epidemiological information, using Adverse Outcome Pathways (AOPs), in order to understand chemical-biological interactions and their consequences on health.
Methods: Literature review and meta-analysis of toxicological data supported by expert judgment were performed to establish: a) adversity pathways, b) quantitative criteria for scoring the observed toxicological effects (adversity indicators), c) NO_2 exposure - adversity relationship for both long-term (1–36 months) and shortterm (1–7 days). The NO_2 daily concentrations from January 2001 to December 2022, were obtained from Madrid city Air Quality network monitoring database. Adversity levels were compared with relative risk levels for all-cause and respiratory mortality estimated using linear equations from WHO 2021 guidelines.
Results: Non-linear relations were obtained for all long- and short-term NO_2 related adversity indicators; for long-term effects, the best fitting was obtained with a modified Haber's law model with an exponential coefficient for the exposure time of 0.25. Estimations are presented for a set of case studies for Madrid city, covering temporal and spatial variability. A clear improvement trend along the two decades was observed, as well as high inter- and intra-station variability; the adversity indicators provided integrated information on the temporal and spatial evolution of population level risk.
Discussion: The proposed HIP conceptual approach offers promising advances for integrating experimental and epidemiological data. The next step is linking the concentration-adversity relationship with population health impacts through probability estimations, the preliminary estimations confirm the need for assessing independently different population groups.

1. Introduction

Nitrogen dioxide (NO_2) is among the regularly monitored air pollutants and in urban environments it is considered an indicator for traffic-related emissions (Atkinson et al., 2018). NO_2 has a shorter atmospheric lifetime than related air pollutants such as fine particulate matter ($PM_{2.5}$), and therefore has more spatial heterogeneity (Wang et al., 2023). WHO and regulatory agencies in different jurisdictions worldwide, such as the European Environment Agency (EEA) and the United States Environmental Protection Agency (EPA), have established air quality guidelines defining levels for acute and chronic exposures to different air pollutants.

The WHO science-based methodology for proposing guideline values has evolved in recent years. The most recent proposal (Geneva: World Health Organization, 2021) assumes that causality is already defined and uses only epidemiological studies (Huangfu and Atkinson, 2020) to set guidelines that are directly linked to the relevant health indicators. It proposes an annual guideline value of 10 μg/m3 and a short-term (24-h) guideline value of 25 μg/m3, with additional interim targets. A main limitation of this approach is the simplification of the concentration-response curve to a linear relationship without threshold, while more specific studies on causal interference suggest non-linear relationships (Dominici et al., 2022). The USEPA Integrated Science Assessment (US EPA, 2016) presents a comprehensive overview of human and animal evidence, and has been used for setting an annual standard of 53 ppb (101 $\mu g/m^3$) and a 1-h standard of 100 ppb (191 $\mu g/m^3$).

Nitrogen dioxide atmospheric levels in many urban areas have decreased in recent years, with an improvement of their health effects (Izquierdo et al., 2020), but are still frequently above the WHO guidelines. This reduction has been linked to specific municipal actions on traffic control and the creation of Low Emission Zones (LEZ), the evolution and improvement of the vehicle fleet and a raising citizen awareness towards a more sustainable mobility. This circumstance exacerbates the limitations of non-threshold linear approaches, triggering the need for developing new alternatives.

On the one hand, experimental toxicity studies offer detailed information on causality and the dose-response, but requires extrapolations, due to the observed effects, in animals or *in vitro*, and the exposure period in the assays to human health impacts. In recent years, system based integrative approaches such as Adverse Outcome Pathways (AOP) and Integrated Approaches to Testing and Assessment (IATA) (Hoffmann et al., 2022a) have emerged to conceptualize the chemical-biological interactions and health consequences at different levels of the biological organization; but the current focus is still on the adverse apical effects observable in the study (Bajard et al., 2023), not moving towards the final health impact. On the other hand, for NO_2 and other air pollutants, there is a significant number of epidemiological information and studies on human volunteers. Therefore, we propose as a proof of concept, a new approach for conceptualizing, the Health Impact Pathway (HIP) summarized in Fig. 1, for integrating the available human and animal studies on NO_2 with probabilistic risk assessment methodologies.

We are using the WHO acute and chronic guidelines as the starting reference, but this baseline can be easily adapted to those relevant for specific jurisdictions, such as the US 1-h standard. Then, the toxicological observations, including studies with human volunteers, are translated into adversity indicators using expert knowledge elicitation and AOP network approaches. It should be noticed that while the conceptualization of an AOP is to be chemically agnostic, the implementation of the AOP for evidence-based methods during the risk assessment process requires chemical specific considerations (Hoffmann et al., 2022b). The health impacts associated to NO_2 exposure are complex multifactorial occurrences. In the proposed conceptualization, adversity indicators should be understood as probabilistic risks, linked to the likelihood of measurable health effects, such as the probability of severe respiratory effects that could trigger hospital admission or even mortality. The concentration-adversity relationship is projected as a progression in the probability of a certain population to develop pathologies ultimately resulting in increases in the population health impact. If verified and implemented, this approach could address some additional shortcomings of current generic guidelines as identified by other authors, such as specific assessments for vulnerable (Boogaard et al., 2023), and disproportionately exposed minority groups including those associated to social inequities (Wang et al., 2023).

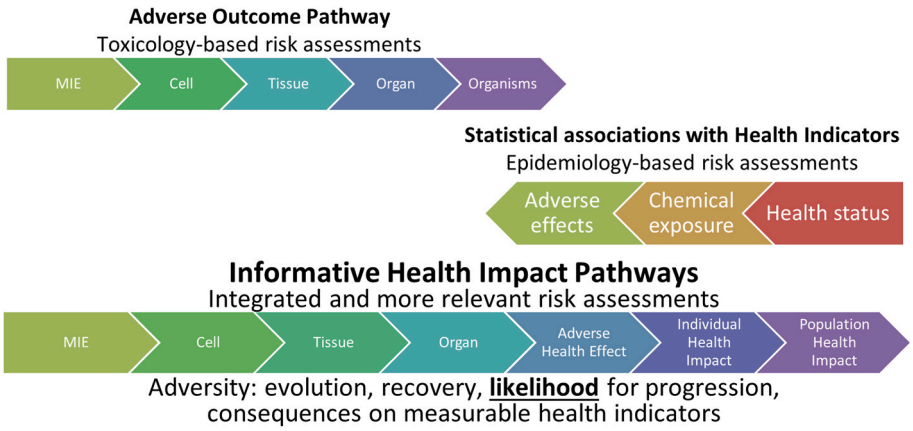

Fig. 1. Proposed conceptual model for linking toxicological and epidemiological results through the extension of the AOP framework as Health Impact Pathway (HIP).

2. Methods

2.1. Data sources and data extraction

Two complementary sources of data were used. The first is the comprehensive reviews conducted by WHO (Geneva: World Health Organization, 2021) and other organizations (Atsdr, 2014.; Canada. Health Canada, 2021; European Environmental Agency, 2022) to establish the guideline values and to provide recommendations. The references included in the cited reviews, were extracted and considered in this assessment selecting the relevant toxicological and epidemiological studies.

The second source was a targeted literature search using PubMed, Scopus and WebOfScience. To cover complement and updated literature search. We used a combination of keywords: "nitrogen dioxide", OR "NO_2", "toxicity", AND "health effects", "human exposure", "animal studies" OR "epidemiology" that covered a wide range of evidence on the health effects and toxicological implications of NO_2 exposure. Search results were filtered to include human clinical studies, epidemiological data and, animal studies. Moreover, the database was extended through a secondary search focused on the references and citations of the selected publications.

The identified references were selected for data extraction using the following inclusion criteria.

1. Defined dose levels: studies had to report concentration levels of NO_2 exposure. The dose values had to be expressed quantitatively (e.g. in parts per million (ppm) or micrograms per cubic meter ($\mu g/m^3$).
2. Information on the exposure period: the studies should clearly state the duration of NO_2 exposure. This includes whether the exposure is continuous or intermittent, the frequency, and the overall period.
3. Well-described effects: studies should provide complete and detailed descriptions of the observed health effects of NO_2 exposure. This includes biochemical changes, physiological alterations, clinical symptoms and observed pathology.
4. Attributable effects: the studies had to demonstrate that the observed health effects were directly attributable to NO_2 exposure, including control groups or other methodological approaches to isolate the effects of NO_2 from other variables.

Not fulfilling one or more inclusion criteria or insufficient information for the verification were set as exclusion criteria.

Results from experimental studies were extracted in an Excel database, each line included the reference, species, exposure concentration (in ppm and $\mu g/m^3$), exposure conditions, equivalent 24-h average exposure, exposure time, observed effects, and other relevant observations. In case several effects were reported for the same exposure conditions, each effect was included in a different line. When the experimental study design included a recovery phase, the column on other relevant observations indicated the observed recovery potential.

2.2. Epidemiological risk indicators

The WHO report on air quality guidelines (AQG) (Geneva: World Health Organization, 2021) provides, for nitrogen dioxide, recommendations for the annual AQG level and three long-term interim targets, based on the review by Huangfu & Atkinson (Huangfu and Atkinson, 2020). The report provides relative risk levels for non-accidental all--cause-mortality and for respiratory mortality for the interim targets, which were used for estimating linear equations between annual average concentrations and relative risk increase. The equations based on annual average guidelines were also used to estimate the concentration-risk index for 12-month moving average values.

The WHO report also includes short-term AQG levels, based on daily (24-h) averages. These are derived on the basis of the 99^{th} percentile of the annual distribution of 24-h average concentrations and include two interim values. The report provides relative risk levels for non-accidental mortality for the interim targets, which were used for estimating linear equations between daily (24-h) average concentrations and relative risk increase.

2.3. Toxicological risk indicators

The data extracted from the toxicological studies, selected as valid and compiled in the excel table (described above), were used in a meta-analysis, to combine the results of the various studies providing evidence on the size of the range of effects caused by NO_2. An Expert Knowledge Elicitation (O'Hagan, 2019) supported by an AOP-network regression modeling approach was conducted for associating the observed effects with an Adversity Index. This approach involves critical evaluation of the evidence, followed by integration of all the information using the AOP conceptual framework, leading to the proposal of an evidence-based AOP network.

The observed adverse effects are then assigned an adversity index, integrating the probability and severity of adverse outcomes. The starting point for the Expert Knowledge Elicitation was to develop the assessment criteria. A list of reported effects in the experimental studies was compiled and, for each effect, the experts discussed and agreed by consensus the associated adversity range using the AOP conceptual framework for ranking the reported effects in an adversity gradient. Then, the criteria were applied to the reported findings, associating an adversity index to each reported effect. The index was within the range

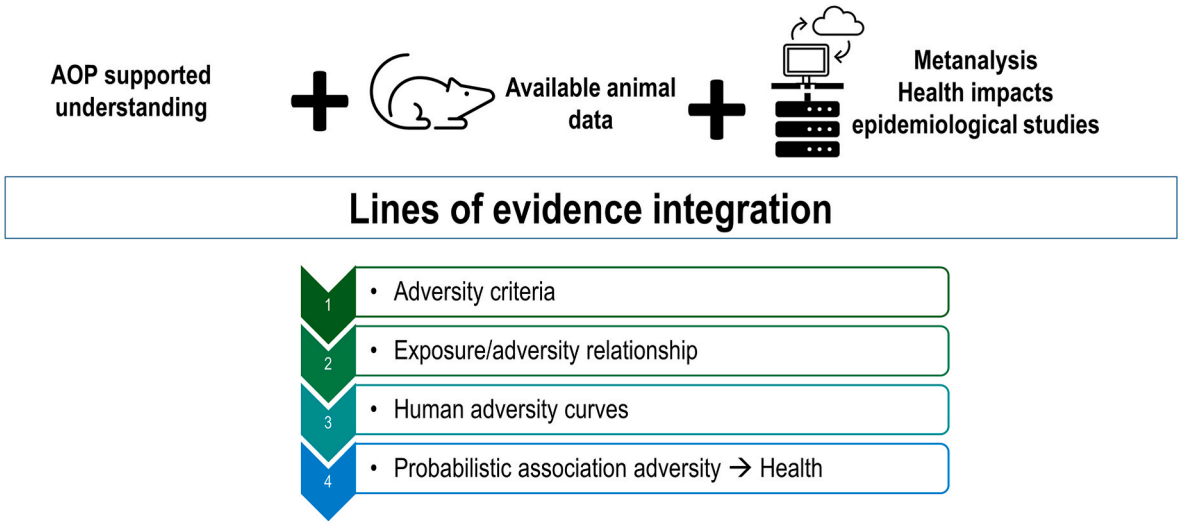

Fig. 2. Summary of the proposed methodology for "Health Impact Pathways" for environmental contaminants.

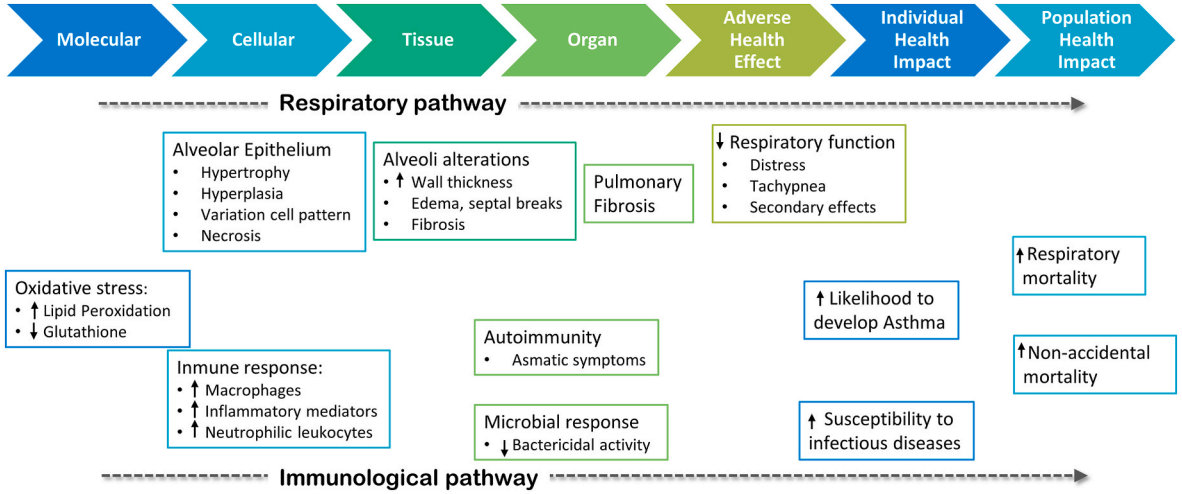

Fig. 3. Proposal of an evidence-based AOP network for the effects of NO2.

indicated in the criteria, and included considerations as incidence, severity, and potential for recovery, following the indications reported in the study publication.

Subsequently, the most severe adversity level for each concentration/exposure association was selected for developing the exposure/adversity relationships, using the exposure times reported in each of the toxicological studies used.

A modification of the Haber's law was applied for integrating the exposure level as a combination of concentration and time, according to the following equation: Adversity Index $= Ct^K$

Were C is the daily average, t the time in days, and K an exponent for correcting the weight of time *vs.* concentration.

The best-fit approach, covering linear or nonlinear models, was used to setting the K-value for the full database, as well as relevant conditions selected after an exploratory assessment for different concentration ranges and time-windows of exposure, both short and long term.

2.4. Selected case studies

A set of case studies were selected using the monitoring database of the municipality of the Spanish capital city Madrid. Open NO_2 ambient air concentration data from January 2001 to December 2022 were extracted as hourly averages from all 24 municipal monitoring stations of the official Air Quality network of Madrid City Council (accessed on March–June 2023) where NO2 is measured. These monitoring stations cover the whole area of the city (see Supplementary Material Fig. 1). Among these 24 monitoring stations, background sites & urban traffic sites are well balanced, representing the most common types of monitoring stations in what respect the characteristics of the surrounding area, far followed in terms of its occurrence by suburban sites. The values were extracted into Excel sheets, curated for errors and inconsistencies, and used for estimating daily, monthly, and annual averages. During the curation process, some periods with no available data due to different reasons (analyzer not working properly, calibration/verification processes, maintenance operations …) were detected, so these periods were not considered for the averaging calculations. The graphical visualization was completed with exploratory statistics using R statistical software for selecting specific case studies covering, long- and short-term temporal variability as well as spatial variability.

The adversity estimation models, i.e the mathematical equations estimating the adversity index associated to each NO_2 exposure level (combining concentration and time), were implemented in Excel for the deterministic estimations and connected to Crystal Ball® for the probabilistic Monte Carlo analysis, where exposure levels and/or equation parameters can be represented as distributions instead of fixed values to cover the variability and uncertainty. The spatial distributions were

Table 1
Agreed adversity criteria for toxicological observations in animal studies.

Category	Event	Adversity score
Early interim events	Increase in ethane exhalation as LP index	0–33
	Increase in content of TBA reactants in lung homogenates as LP index	0–33
	Decrease in Glutathione peroxidase (GPx) activities in the lungs	0–33
Histopathological alterations	Hypertrophy and loss of cilia in Bronchial epithelium	35–40
	Hypertrophy, hyperplasia, metaplasia and devoid of cilia in bronquiolar epithelium	45–50
	Variation of epithelial alveolar type I cells pattern	35–50
	Increase in bronquiolar nonciliated secretory (CLARA) cells number.	45–50
	Necrosis of epithelial alveolar cells type I and variation of type II cell pattern	40–50
	Cytoplasmatic alterations and necrosis of epithelial alveolar cells type II	40–50
	Increase of alveolar macrophages in terminal bronchioles and proximal alveoli	45–55
	Macrophages releasing inflammatory mediators	45–55
	Thickening in of walls in the area through the bronchopulmonary junction to the alveolar duct	40–60
	Increases in arithmetic mean thickness (AMT) of the alveolar wall.	40–60
	Bronchiole-alveole junction totally or partially blocked with cellular debris.	60–75
	Pneumonitis, peribronchial lymphocytic infiltration (PLIP). Bronchiolar epithelial erosion.	60–80
	Increased neutrophilic leukocytes (PMNs)	40–55
	Interstitial edema of alveolar septa. Alveolar spaces lost normally homogeneous pattern	55–65
	Interstitial edema replaced by collagen fibers. Interstitial fibrosis.	60–85
	Alveoli distended, alveolar septal breaks	50–85
	Reduction in the number of alveoli and ventilatory surface reduced accordioning	60–90
	Polycythemia	45–55
	Cardiac hypertrophy related to polycythemia and dehydration	60–75
Immunological alterations	Significant decrease in pulmonary bactericidal activity	40–75
Clinical signs	Respiratory distress	70–85
	Tachypnea	70–80
	Increased respiratory symptoms in asthmatic individuals	70–85
Mortality		100

assessed using QGIS, where Inverse Distance Weighting (IDW) interpolation was employed to estimate adversity values based on air quality monitoring stations placement using a distance coefficient of 2 and a pixel size of 10.

A schematic representation of the steps followed in this methodology is shown in Fig. 2.

3. Results

3.1. Epidemiological risk indicators

The concentration/risk equations obtained from the WHO guidelines were:

$$\text{Relative Long-Term Risk all mortality} = 0.2AAC + 98 \quad [1]$$

$$\text{Relative Long-Term Risk Respiratory mortality} = 0.3AAC + 97 \quad [2]$$

$$\text{Relative Short-Term Risk all mortality} = 0.0731DAC + 98.25 \quad [3]$$

where AAC and DAC represents the Annual and Daily Average Concentrations, respectively.

It should be noted that the scales for long- and short-term relative risks are different, as WHO uses the same approach (relative risk of 100) but different baselines; therefore, the long-term risk is relative to an annual average concentration of 10 μg/m^3, while the short-term risk is relative to a daily average concentration of 25 μg/m^3.

3.2. Toxicological risk indicators

The metanalysis of experimental animal studies included 89 datasets from eight different publications; 26 datasets covered immunodepression effects observed in "challenge studies" with infectious agents, and the others covered a broad variety of toxicological endpoints, from biochemical alterations to histopathological and clinical effects, including lethality. Fig. 3 shows the proposed evidence-based AOP network that schematically integrates all information using the AOP conceptual framework.

Table 1 summarizes the agreed criteria employed for translating the effects into an adversity index.

The analysis of the exposure/adversity relationships using the classic Haber's law (K = 1) identified two main datasets, one for exposure levels below 1000 μg NO_2/m^3 and one for higher exposure concentrations. The best fit exercise confirmed the need for including a K exponent in the concentration/time exposure integration, and that the K exponent was concentration dependent, with lower values for lower concentration levels, indicating a lower relative weight for exposure time at lower exposure concentration. It should be noted the inequality of the adversity data, as the dataset for lower concentrations is based on medium to long-term exposure times (from 3 months to over 2 years), while the data set for higher concentrations also include short-term (one or few days) exposure times. The best fit ($R^2 = 0.92$) for the effects observed at average concentrations below 1000 μg NO_2/m^3 was obtained for a K coefficient of 0.25. Table 2 summarizes the selected adversity values and the rationale for the adversity valuations. In line with standard risk assessment approaches an interspecies extrapolation factor of 10 was applied to translate the adversity observed in the experimental animals to adversity estimations for humans, resulting in the following equation:

$$\text{Human Adversity Index} = 15.2\ln(Ct^{0.25})\text{-}13.8 \quad [4]$$

Where C is the NO_2 concentration expressed as daily average and t the exposure time in days.

The extrapolation is based on a dataset covering medium to long-term exposure times (from 3 months to over 2 years), and therefore was considered appropriate for the estimation of human adversity linked to long-term exposures, such as annual averages. For this timeframe, the annual concentration values covered by actual data includes the range of 10–100 μg NO_2/m^3, covering the actual values measured in many urban areas worldwide. The estimated human adversity threshold for annual average concentration was 2.4 μg NO_2/m^3.

Most experimental acute toxicity studies focus on lethality or highly severe effects. However, the study by Rombout et al. (1986) included three parallel designs covering short-time exposures and was selected for setting the adversity/exposure relationships for short-term exposures (see Supplementary Material Table S1). The dataset includes continuous, i.e. 24-h per day, and intermittent, 6-h per day, exposures, the second resembling occupation conditions. For exposures up to 16 days, full concordance between the level of adversity observed for continuous

Table 2
Effects and adversity levels assigned in toxicological animal studies after exposure to low dose of NO2 and different exposure times.

Study	Spp	Concentration		Exposure				Adversity level[a]	Effects
		ppm	ug/m3	daily fraction	daily average	days	months	score	
Kubota et al. (1987)	rat	0,04	76,6	1	76,6	270	9	35	Tendence of thickening in of walls in the area through the bronchopulmonary junction to the alveolar duct
	rat	0,4	765,6	1	765,6	270	9	40	Same observations as at 0.04 ppm with increased severity.
	rat	0,04	76,6	1	76,6	540	18	40	Slight bronquial hipertrophy. Variation of alveolar type II cells pattern
	rat	0,4	765,6	1	765,6	540	18	45	Bronchial hipertrophy. Evident thickening of alveolar duct walls. Increase in CLARA cells number.
	rat	0,04	76,6	1	76,6	810	27	40	Morphometric indices showing tendence of increment of AMT, interstitial components.
	rat	0,4	765,6	1	765,6	810	27	80	Alveolar epithelium alteration confirmed by ME. Interstitial edema preplaced by collagen fibers. Interstitial fibrosis.
Blair et al. (1969)	mice	0,5	957,0	0,25	239,3	90	3	60	Pneumonitis, peribronchial lymphocytic infiltration (PLIP). Alveolar septal breaks and expansion.
	mice	0,5	957,0	0,75	717,8	90	3	60	Pneumonitis, peribronchial lymphocytic infiltration (PLIP). Alveolar septal breaks and expansion.
	mice	0,5	957,0	1	957,0	90	3	70	Pneumonitis, obstruction on some bronchioles and devoid of cilia in epithelium. Alveolar septal breaks and expansion.
	mice	0,5	957,0	0,25	239,3	180	6	60	Pneumonitis, peribronchial lymphocytic infiltration (PLIP). Alveolar septal breaks and expansion.
	mice	0,5	957,0	0,75	717,8	180	6	70	PILP ↑, devoid of cilia in bronchial epithelium. Septal breakage. Bronchiole-alveole junction totally or partially blocked with cellular debris.
	mice	0,5	957,0	1	957,0	180	6	75	PILP ↑↑, devoid of cilia in bronchial epithelium. Septal breakage. Bronchiole-alveole junction totally or partially blocked with cellular debris.
	mice	0,5	957,0	0,25	239,3	270	9	55	Pneumonitis↓. Thicker alveolar septum, minimal inflammation and expansion of alveoli.
	mice	0,5	957,0	0,75	717,8	270	9	55	Pneumonitis↓. Thicker alveolar septum, minimal inflammation and expansion of alveoli.
	mice	0,5	957,0	1	957,0	270	9	55	Pneumonitis↓. Thicker alveolar septum, minimal inflammation and expansion of alveoli.
	mice	0,5	957,0	0,25	239,3	365	12	60	Moderate pneumonitis, PILP. Bronchiolar epithelial erosion.
	mice	0,5	957,0	0,75	717,8	365	12	75	Severe pneumonitis. Bronchiolar epithelial erosion.
	mice	0,5	957,0	1	957,0	365	12	80	Severe neumonitis, some lung lobes in stage of gray hepatization. Exudate in alveoli and infiltration of polymorphonuclear leukocytes.

[a] Adversity level score: (0–33): early interim effects; (34–99): General events; (100): Mortality.

and intermittent exposures was found when the exposure concentration is estimated as daily average (Haber's law with no correction factor). The dataset is limited to two concentrations with several timepoints, similar findings were observed for each concentration, with the slope of the time/adversity curve decreasing after 7 days reaching a semi-plateau situation. The concentration/adversity curves for 2 and 7 days suggested a log-linear relationship with a common threshold of 10 μg NO_2/m^3. This approach was extended to the 1 day exposure for which effects were reported only at the highest concentration. Applying the interspecies extrapolation factor of 10 to translate the adversity observed in the experimental animals to adversity estimations for humans, the following equations were obtained:

$$24h\_Human\ Adversity\ Index = 17.4\log_{10}(24h\ average\ concentration) - 17.4 \quad [5]$$

$$48h\_Human\ Adversity\ Index = 22.1\log_{10}(48h\ average\ concentration) - 21.6 \quad [6]$$

$$7d\text{-}Human\ Adversity\ Index = 32.5\log_{10}(7d\ average\ concentration) - 32.6 \quad [7]$$

All with a threshold of NO_2 10 μg/m^3. Table 2 summarizes the selected adversity values and the rationale for the adversity valuations. Fig. 4 presents the proposed exposure/adversity curves for long- and short-term NO_2 exposures.

3.3. Case study 1: adversity estimations for annual average concentrations

For this first case study, we selected two monitoring stations to exemplify the capacity of long-term adversity estimations: Escuelas Aguirre (high traffic intensity) and Casa de Campo (low traffic intensity). For each station, daily average concentrations for the full selected period (January 2001 to December 2022) were used for estimating annual average concentrations and 3, 6, 12, 24 and 36 months running-average concentrations. Relative Risks for all- and respiratory-mortality were estimated for the annual average concentrations, according to equations [1] and [2] described above. Human Adversity Levels linked to exposures from 3 to 36 months were estimated from the running-average concentrations according to equation [4]. The results are presented in Fig. 5. Subfigure A presents the evolution of NO_2 monthly average concentrations; B the relative risks for each station according to the WHO proposal, while C and D represent the evolution of the Human Adversity Level for each station, solid lines represent the timelines covered by the toxicity studies, 3–24 months, dotted lines the extrapolation to shorter and longer periods. The adversity estimations are non-linear, offering information on the expected health impact of the concentration evolution. In addition, the possibility for estimating expected adversity levels for different timeframes provides enhanced assessments for understanding the temporal evolution. As an example, it can be observed that in the high traffic intensity station (Fig. 5. Subfigure C), the adversity level always increases when considering longer

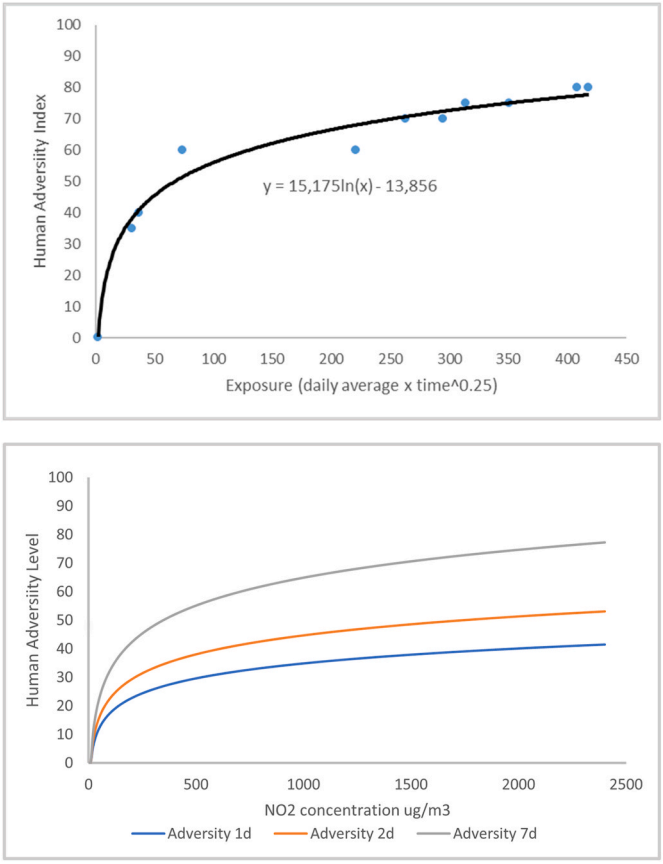

Fig. 4. Exposure/adversity curves for long- and short-term NO_2 exposures.

timeframes, while in the low traffic intensity station (Fig. 5. Subfigure D) the adversity is sometimes higher for timeframes shorter than a year.

3.4. Case study 2: adversity estimations for daily average concentrations

We selected the same monitoring stations to exemplify the capacity of short-term adversity estimations. Equations (5)–(7) have been used to calculate the evolution of the adversity estimations during 2019, the results are presented in Fig. 6. Both stations are characterized by a generic "U" pattern for NO_2 concentrations, with lower values in summer but with a high daily variability. It should be noted a similar trend for short-term trends than that observed for the long-term patterns. In the high traffic intensity station, the adversity level always increases when considering longer timeframes, while in the low traffic intensity station the adversity is sometimes higher for timeframes shorter than a week.

3.5. Case study 3: spatial distribution of adversity values

Fig. 7 presents the integration of all Madrid city monitoring stations into spatially explicit representations of the adversity levels. The translation of concentrations into adversity levels offers additional information for managing risks, particularly for the short-term assessment as the adversities integrate the concentration evolution from previous days. This information facilitates the identification of areas requiring further interventions, but also the identification of the city areas with the highly exposed groups. The spatial evolutions complement the probabilistic assessments and the definition of realistic exposure levels according to citizens' moving patterns (see supplementary material).

4. Discussion

Toxicology and epidemiology address the effects of chemicals, such as environmental pollutants, from different perspectives. Both should be considered as complementary and mutually supportive approaches, despite the need for reconciliating divergent results in some cases (Andersen et al., 2019). For setting NO_2 AQGs, WHO initial assessments included both considerations, but the more recent ones have focused on epidemiological studies only. Establishing acceptable quality guidelines (AQGs) is a crucial first step. However, when the recommended levels are exceeded, a risk assessment must be carried out to quantify the actual risk at the population level. This involves two stages, according to the risk assessment paradigm: a) estimating realistic exposure levels based on concentration-time distributions using monitoring data; and b) generating exposure-response relationships for pertinent timeframes.

In this study, we have proposed a new approach that shows its usefulness in several case studies for the city of Madrid. The conceptual approach—integrating human and animal data using AOPs—shares many similarities with the US EPA's integrated scientific assessment (US EPA, 2016). However, the ultimate goal is to establish a quantitative exposure-adversity relationship, which necessitates further efforts to integrate the experimental data, rather than to suggest a standard or point of departure for determining the acceptable level.

Previously, several researchers and organizations have proposed different alternatives and frameworks for integrating toxicological and epidemiological results as lines of evidence for chemical risk assessments, covering different chemical groups such as pesticides (Pelkonen et al., 2019) as well as the assessment of mixtures (Hernandez et al., 2019). It should be noted, that in these case toxicological studies were the basis for setting guideline values, and epidemiological studies considered as supportive information for validation.

Toxicological studies in animal models, conducted under well-characterized exposure conditions, provide strong lines of evidence, although in many cases the apical effects are observed at higher concentrations than in the environment. For example, a recent review concluded that harmful effects of persistent organic pollutants are observed at higher exposures than those determined in environmental studies (Muir et al., 2022). In addition, toxicological studies, when assessed from a mechanistic perspective, and not only considering the final endpoint, can provide very useful data.

The use of AOP approaches has been proposed for integrating toxicological results in the assessment of ambient particulate matter (Li et al., 2022; Wang et al., 2022). Main elements when using AOPs in risk assessment (Hoffmann et al., 2022a) include the availability of data and the need for prioritization. In addition, the effects measured in animal studies are very different from the health indicators used for setting guideline values for atmospheric pollutants from epidemiological studies. The approach proposed in this proof of concept is that the animal studies can be translated into adversity levels. Results are grouped into adversity categories, based on an AOP-supported mechanistic understanding, building exposure/adversity relationships. A key element of this approach is that adversity should be interpreted in probabilistic terms. The observed/expected effect may progress leading to more severe health impacts, may be functionally compensated by the individual, or even fully recovered through repair mechanisms. Therefore, the adversity cannot be interpreted as certainty for progressing towards health impacts, but as increased likelihood, i.e., for each population group, the probability of the final health impact is a function of the adversity levels measured at different timeframes and the specific susceptibility of the group. This information can be used to translate exposure measurements into relative population risks using the proposed HIP approach. This is particularly relevant for atmospheric pollutants covered by comprehensive monitoring programs, such as NO_2. The implementation should be adapted to the available information and simplified in line with the assessment needs.

This approach requires flexibility in the adaptation of the adversity

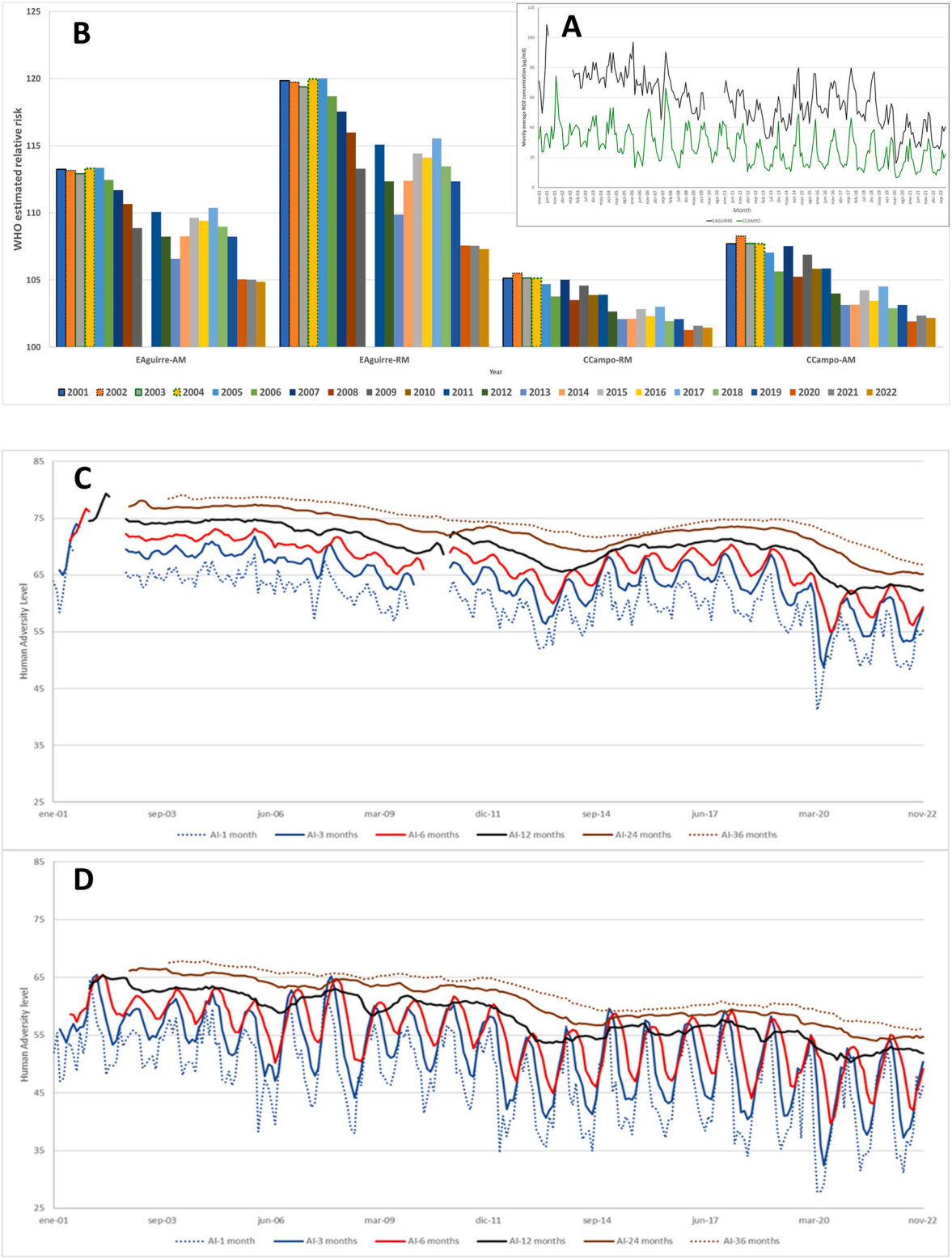

Fig. 5. Evolution of NO_2: Long-term related adversity since 2001 in air quality monitoring stations of Madrid with high (Escuelas Aguirre) and a low (Casa de Campo) traffic intensity. A - Monthly averaged NO_2 concentrations; B – Estimated WHO relative risks; C - Long-term (1–36 month) adversity for Escuelas Aguirre; D -. Long-term (1–36 month) adversity for Casa de Campo.

pathway, as some effects are linked to more than one level in a conventional AOP. For NO_2, the first relevant effects are epithelial responses, at alveolar and bronchiolar levels, which should be considered as cell/tissue responses. At increased exposure (concentration/time) levels, the initial alterations evolve into tissue/organ structural alterations and then functional adverse effects impacting individual health. In parallel there is an initial immune related cellular response that may evolve into autoimmunity, increasing the likelihood for developing asthma and related health problems, and/or decreased capacity to respond to microbial challenges increasing the susceptibility to

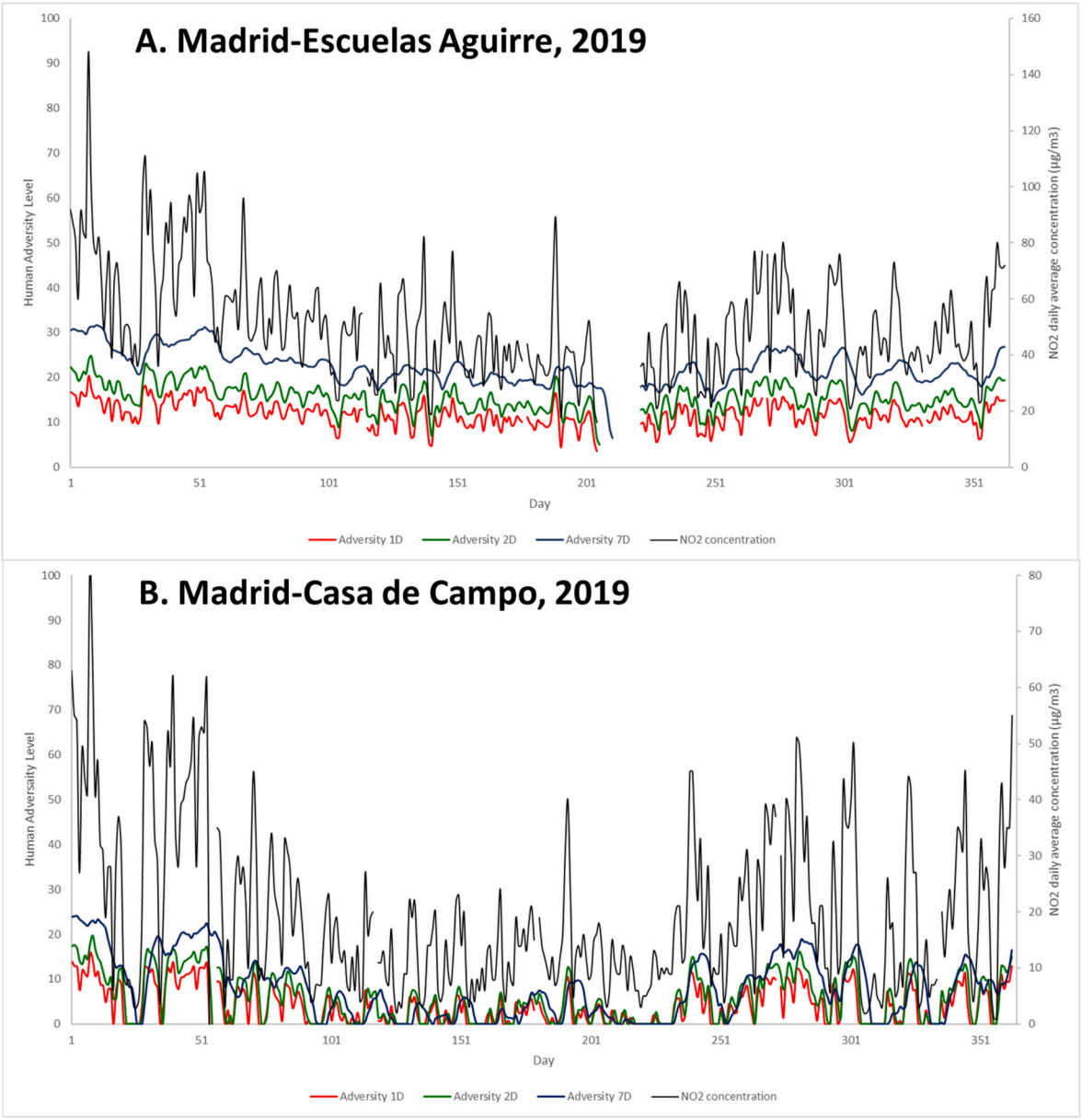

Fig. 6. Evolution of NO_2 related short-term adversity estimations during 2019 in air quality monitoring stations of Madrid with high (A-Escuelas Aguirre) and a low (B-Casa de Campo) traffic intensity.

infectious diseases (Fig. 3). These three adversity lines may result in population health impacts, connected to the increased rate of respiratory and non-accidental mortality described in the epidemiological studies.

As exemplified in the case studies using available open data for Madrid (Spain), actual NO_2 exposure in urban environments is highly variable, despite long- and seasonal-trends. The proposed approach offers risk managers the possibility to assess the adversity of NO_2 exposure at various timeframes, from one day to several years. In these case studies, we have used the same adversity criteria for short- and long-term timeframes. This is on purpose as this way risk managers may assess the actual temporal trends and establish the most relevant timeframes for assessing health impacts and the consequences of specific interventions. Similarly, to many other large cities, Madrid municipality has implemented specific measures for reducing urban atmospheric pollution (Salas et al., 2021). A previous study estimated the expected health impacts using relative risk ratios from epidemiological studies (Izquierdo et al., 2020); and similar studies are available for other cities and even at global level (Song et al., 2023). A complex network connects urban/transport planning with health impacts (Giles-Corti et al., 2016), and air pollution is frequently identified among the most important factors (Donzelli and Linzalone, 2023). NO_2 is not only a toxic atmospheric pollutant, but also a traffic intensity indicator and a precursor of other pollutants such as O_3. Despite the relevance of epidemiological studies, the vehicles' fleets, renewal towards electric vehicles and those with reduced emissions, represents an additional difficulty for using epidemiological metanalysis for associating NO_2 levels with actual and future health impacts. Our proposal offers complementary information in the form of health adversity estimations for different timeframes, where adversity is directly and exclusively linked to NO_2 exposure. These adversity models can be used for supporting the interpretation of epidemiological studies. In particular, when conducting a metanalysis of different epidemiological studies conducted in different locations or different time periods, the estimation of the adversity indexes associated to the measured concentrations could support the understanding of the differences observed between studies, providing a common toxicological baseline for the measured concentration, and for the temporal and

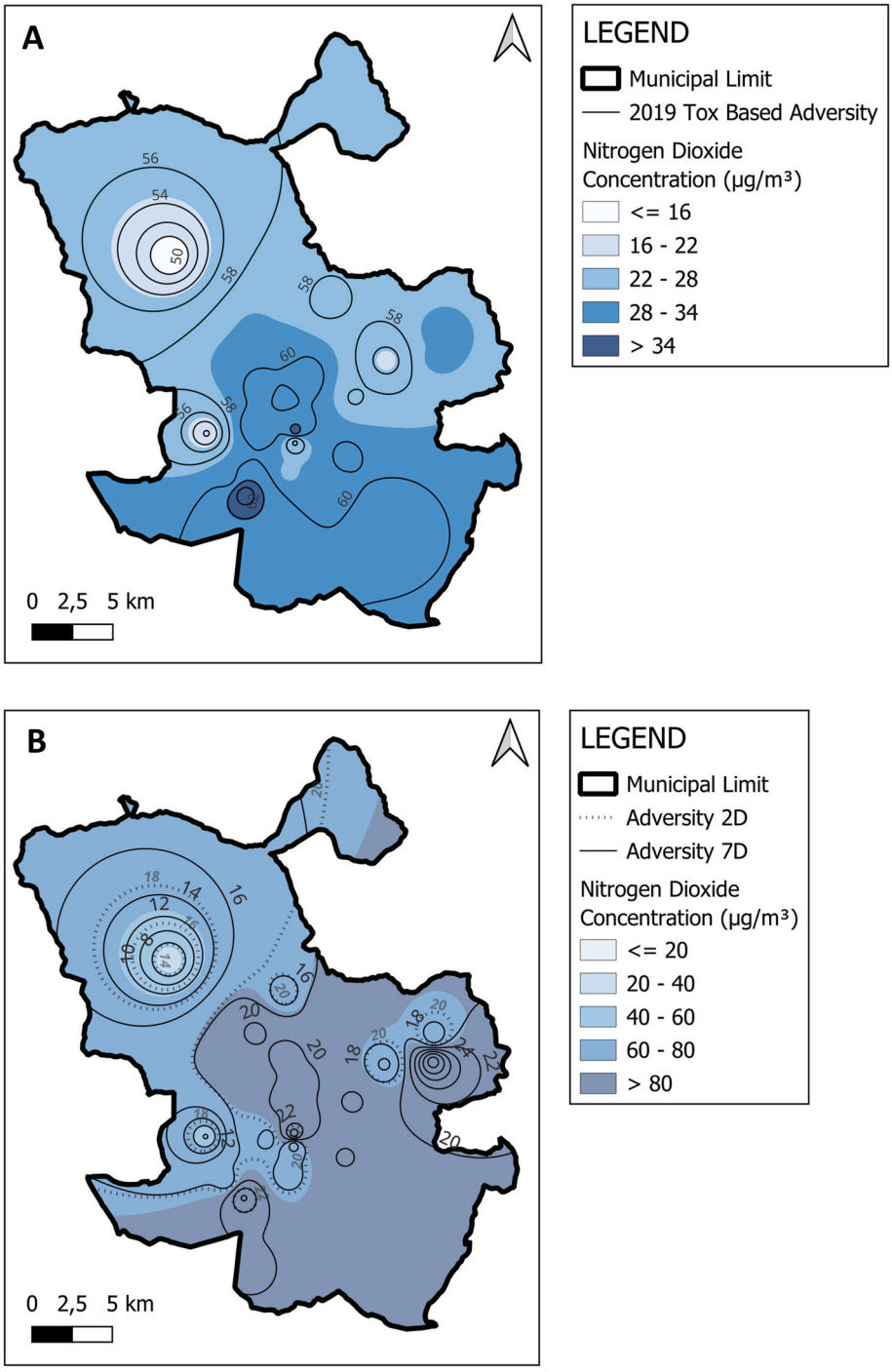

Fig. 7. Spatial evolution of NO_2 related adversity in Madrid city. (A) Long-term, annual average for 2019, and (B) Short-term, two- and seven-days average for February 5, 2019.

spatial variability of the measured levels.

As for any proof of concept, there are obvious limitations to this approach and needs further refinement and discussions within the scientific community and with stakeholders for facilitating its implementation. The first limitation for the NO_2 case study is the limited availability of relevant toxicological data, and the need for animal to human extrapolation. The assumption of higher sensitivity for humans than rodents is supported by comparison of toxicological animal studies and those with human volunteers (US EPA, 2016.). We are proposing a generic factor of 10, used for standard risk assessments, as the information is limited for conducting a proper quantitative estimation. The second limitation, is the comparison of exposure timeframes between humans and animals. It is partially covered by the use of a modified Haber's law, as the dataset covers a wide range of exposure conditions, we have used regression analysis for the full and different subsets and the assumption of an exponential relationship seems reasonable. A related issue requiring further discussion is the comparison of exposure patterns and species life expectancies, as exposures of one/two years represent a short timeframe for human development but the full lifetime for experimental rodents, this is part of the epistemological uncertainty associated to animal to human extrapolations.

The most relevant application of the proposed approach is through

the development of probabilistic functions linking the adversity indicators with the health indicators used in epidemiological studies. Some conceptual examples are presented (see supplementary material) exploring this opportunity, but the proposal is to move this proof of concept to more relevant estimations, assessing the combined health impacts related to the exposure to several atmospheric pollutants. This requires first, the development of similar approaches for other atmospheric pollutants such as ozone, sulfur dioxide and particulate matter, using animal toxicity studies and human volunteer studies with well characterized exposure conditions, the consideration of combined adversity relationships for several pollutants using AOP networks, and finally the integration with epidemiological studies. This is part of an ongoing research line exploring the development of HIPs as part of the Next Generation Risk Assessment (NGRA) developments (Schmeisser et al., 2023).

CRediT authorship contribution statement

Susana Pallarés Porcar: Writing – original draft, Visualization. **Francisco Javier Sánchez-Íñigo:** Writing – original draft, Visualization. **Beatriz Nuñez-Corcuera:** Writing – review & editing, Investigation. **Joaquín Lozano Suárez:** Writing – review & editing, Visualization. **Sonia Arca-Lafuente:** Writing – review & editing, Investigation. **Clara Moyano Cárdaba:** Writing – review & editing, Investigation. **Ana Fernandez Agudo:** Writing – review & editing, Investigation. **Mercedes de Alba-Gonzalez:** Writing – review & editing, Visualization, Investigation. **Rebeca Ramis:** Writing – review & editing, Investigation. **David Galán-Madruga:** Writing – review & editing, Investigation. **Maria del Carmen González-Caballero:** Writing – original draft, Visualization, Supervision. **Verónica Briz:** Writing – review & editing, Investigation. **Susana Guevara-Hernandez:** Writing – review & editing, Investigation. **Ma Encarnación de Vega Pastor:** Writing – review & editing, Investigation. **Denis Sarigiannis:** Funding acquisition. **Saul Garcia Dos Santos:** Writing – review & editing, Funding acquisition. **Jose V. Tarazona:** Writing – original draft, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.142883.

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