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Fabrice Marliere

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**ACUTE TOXICITY OF SMOKE : STUDY OF GASEOUS INTERACTIONS**

*INERIS - Parc Technologique ALATA - B.P. n° 2*

*60550 VERNEUIL EN HALATTE - France*

**F. MARLIERE**

**ABSTRACT**

A previous paper entitled " Toxicity of fire smoke" and presented at the European workshop held in Cadarache (Industrial fires II, 1994) described the first phase of a two phases project supported by the French Ministry of Environment. This first phase was dealing with the contribution of smoke particulates (soots + aerosols) in acute toxicity and, more precisely in the degree of incapacitation, by using an original behavioural study of exposed animals (Wistar rats).

The present paper concerns the second phase of the project. It is carried out entirely in laboratory and comprises a behavioural post-exposure study considering the degree of incapacitation. It consists in the reconstitution of toxic atmospheres in laboratory from synthetic gases. Selected gases (CO, NO, NO<sub>2</sub>, HCN, SO<sub>2</sub>) are narcotics or irritants, so different types of combinations can be done. Oxygen and carbon dioxide levels are kept constant respectively to 19 % and 1,5 %. An inhalation installation has been designed so as to obtain toxic mixtures of known composition held constant for 30 minutes and to permit animals to be exposed.

The purpose is to evaluate the different types of interactions (synergy, antagonism, additivity) occurring during inhalation of non-lethal gas mixtures. These interactions are revealed on diagrams obtained by computation treatment of behavioural data (descriptive method) which gives a general view of the degree of incapacitation for each tested atmosphere, and involved interactions. Revealed interactions are expressed in term of "tendency" and are quite well correlated with animals clinical follow-up and some biological analysis ( blood gases for example). Main results and conclusions are presented and show that toxic interactions are a complex subject which need to be further investigated (in a biochemical way for example) in order to identify physiological mechanisms involved in interactions.

## . INTRODUCTION

In the event of a fire, the formation and the dispersion of a plume containing toxic components is one of the major issues (among other things). The consequences can be serious for the environment and more especially for population living in the surrounding.

Two types of exposures to toxic fire smoke are to be considered in the assessment of the toxic impact of smoke study :

- the first relates to the confined environment. Fire-fighters and trapped people are concerned by this exposure to undiluted effluents, so to high concentrations of toxic components.

- The second type relates to the neighbouring and populations located at a distance of 50 meters to several kilometers from the fire place. These people are exposed to the cloud issuing from the dispersion of the plume of smoke in the atmosphere, so to relatively low concentration levels. Even in that case, inhalation of the polluted air may be an actual hazard.

Acute toxicological consequences of these inhalations are different and range from lethality to nausea or headache for example.

Studies dealing with the improvement of our knowledge of the toxic effluents of fires have enabled considerable significant progress to be made in areas regarding the emission of gases and their significance in actual fires, with the surveying of toxic components and their mechanisms of formation, animal experimentation techniques, the respective effects of fire gases,...

Among these research fields which must be continued, effects of toxic gases remain an important subject of discussion. It is still required to perfect our current knowledge of the interactions between gases (synergetic, additive or antagonistic effects observed on exposed organism) and consequences ensuing from a short time exposure. The toxic effects of the inhalation of individual component or gas mixtures can be studied from different points of view, either through the intermediary of biological responses obtained from the exposure of in-vivo or in-vitro models, or by behavioural responses of in-vivo models. Each of these different methods provides precious information. However, the high costs of these studies means that very few research programmes focussing on the measurement of the toxic impact include these different aspects at the same time and it is therefore difficult to correlate and compare these data issuing from different origins. More generally, the limited scope of the data prevents from reflecting a complete picture of involved phenomena.

It was with this feeling in mind that INERIS (Institut National de l'Environnement industriel et des RISques, France) developed a programme financially supported by the French Ministry of Environment. Started in 1991, it studied firstly the toxic impact of smoke of full scale fire tests involving rats as animal model. These results were briefly presented at Cadarache (Industrial Fires II, 1994). The second phase, ended in 1995, studied toxic impact of gas mixtures inhalation by using a behavioural method developed and improved during the first phase, and carried

out biological investigations in order to identify some early indicators of intoxication. This paper presents the behavioural part of this study.

## **. OBJECTIVES**

In a first time, the objectives of the study were to perform animal sublethal exposures of 30 minutes enable to reveal the toxic effect of single narcotic and irritant gases. Selected gases were carbon monoxide (CO) and hydrogen cyanide (HCN) as narcotic gases, sulfur dioxide (SO<sub>2</sub>) and nitrogen oxides (NO<sub>x</sub>) as irritant gases. NO<sub>x</sub> effect was considered mainly irritant and they were used as unique gas. In a second time, more or less complex mixtures were realized by binary, ternary,...combinations of selected gases in the aim to observe interactive effects. NO<sub>x</sub> components, nitrogen oxide (NO) and nitrogen dioxide (NO<sub>2</sub>), effects were succinctly studied.

Gases concentration were adjusted by reference to full scale test results and adequate scaling factor.

Toxic impact was assessed through the intermediary of a behavioural test giving results in term of degree of incapacitation.

This was completed by a clinical follow-up of exposed animals, blood gases analysis (CO and CN<sup>-</sup>) and biochemical investigations (not discussed here).

## **. EXPERIMENTAL**

### **Animal exposure device**

The animal exposure device used all long the inhalation programme and enabling known concentration mixtures to be made is shown in figure n°1. It is entirely set under a large hood.

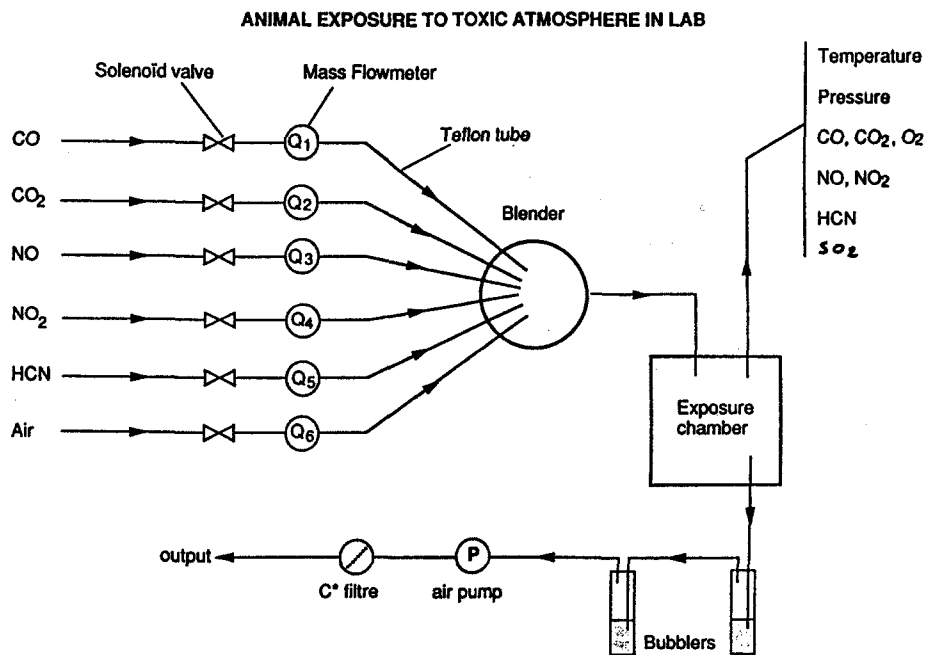
This device comprises a specific Teflon line for each gas (included air of dilution). Each gas flow is controlled by a mass flow-meter and a solenoid valve is inserted in the line for safety reasons.

Concentrated gases are stored in pressurized cylinders :

- CO	4129 ppm in air
- CO <sub>2</sub>	19,25 % in l'air
- NO	4920 ppm in N <sub>2</sub>
- NO <sub>2</sub>	533 ppm in air
- HCN	879 ppm in air
- SO <sub>2</sub>	1800 ppm in air

Whatever the mixture is, toxic gases flows are very low compared to the total flow passing through the exposure chamber. The air flow represents more than 90% of the total flow which is about 1500 l/h.

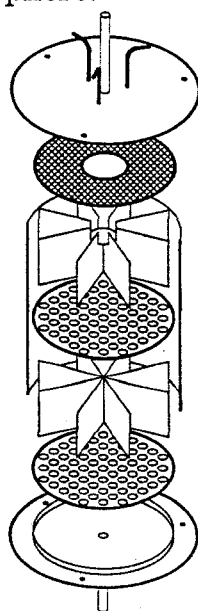
Each line is connected to the blender which enables mixtures to be homogenized.



**Figure 1 : animal exposure device**

Once homogenized in the blender, gas mixtures reached the exposure chamber shown in figure n°2. It is designed as a cylindrical volume of 20 liters, divided in two levels, each level being itself divided in 8 compartments. Physico-chemical analysis are performed inside the exposure chamber in order to characterize the actual exposure conditions. These conditions are characterized in terms of ambient temperature measurements at both levels, internal relative pressure, on-line analysis for CO, CO<sub>2</sub>, O<sub>2</sub>, NO, NO<sub>2</sub> and HCN. All information are collected and centralized on a PC computer, and are thus immediatly accessible.

When they leave the exposure chamber, gas mixtures flow through 2 bubblers filled with soda solution in order to absorb soluble gases, then through a pump to balance relative pressure inside the device, and finally through an active carbon filter set before being discharged to the atmosphere.



**Figure 2 : Animal exposure chamber**

## Test mixtures

The composition of test mixtures is presented in table 1. Each mixture contains CO<sub>2</sub> at the concentration level of 1,5 % in order to keep the hyper-ventilation effect influence constant, and O<sub>2</sub> level is kept at the concentration of 19 % minimum in order to avoid hypoxia phenomena. Groups n°1 and 13 are exceptions to these rules because they are control animals exposed to ambient air.

Group	CO	NO	NO <sub>2</sub>	HCN	SO <sub>2</sub>
1	-	-	-	-	-
2	-	165 ppm	-	-	-
3	-	-	60 ppm	-	-
4	600 ppm	-	60 ppm	90 ppm	-
5	600 ppm	165 ppm	60 ppm	90 ppm	150 ppm
6	-	-	-	-	150 ppm
7	-	165 ppm	60 ppm	90 ppm	150 ppm
8	600 ppm	-	-	90 ppm	150 ppm
9	600 ppm	165 ppm	-	90 ppm	-
10	-	165 ppm	60 ppm	-	150 ppm
11	600 ppm	-	-	-	150 ppm
12	-	-	-	90 ppm	150 ppm
13	-	-	-	-	-
14	600 ppm	-	-	-	-
15	-	-	-	90 ppm	-
16	-	165 ppm	60 ppm	-	-
17	600 ppm	-	-	90 ppm	-
18	600 ppm	165 ppm	60 ppm	-	-
19	-	165 ppm	60 ppm	90 ppm	-
20	600 ppm	165 ppm	60 ppm	90 ppm	-
21	600 ppm	165 ppm	60 ppm	-	150 ppm

Table 1 : mixtures composition

## Animal model

Exposed animal were WISTAR ICO/WI male rats. Eight animals by group were required for each toxic atmosphere exposure. The same animals were biochemically investigated just after the end of the behavioural test. The sole control animal groups exposed to air required 10 rats.

## Exposure conditions

Animals were exposed during 30 minutes to toxic atmospheres at constant concentration and ambient temperature. During exposure, animals were followed visually to set up the clinical follow-up. At the end of the exposure, animals were immediately transferred into their individual behavioural test device.

## . BEHAVIOURAL STUDY

### Principle

Toxic effects of smoke and soots produced in a fire can be revealed by the analysis of behavioural variations of exposed animals.

The protocol proposed is to reveal and measure behavioural signs which are characteristic of inhaled mixtures while carrying out a previous learnt task.

It looked judicious to reveal these impacts when the animals are placed in an emergency situation. This situation is here to get food.

The experimental device used, called Skinner box, is presented on figure 3. It comprises different areas usefull in data treatment. There is the cup area (C), the lever area (L), the round-trip area and the "elsewhere" area. The animals move freely inside this device. Limits of areas are virtual. Sidewalls are made of plexiglas.

To get food, animals are trained to push on the lever with sufficient vertical force to induce a food pellet delivery in the cup.

A well-conditioning is considered achieved when the animals stay within areas which enable them to get food. The "elsewhere" area has no interest for such an animal.

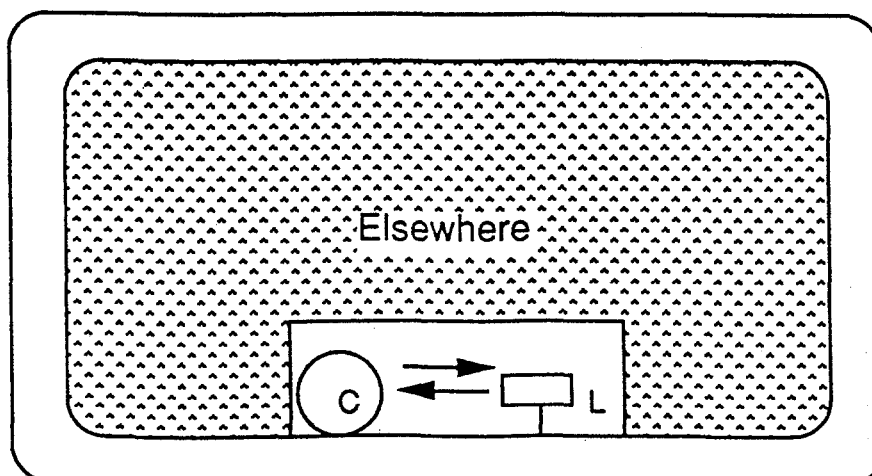


Figure 3 : Skinner box

Animal conditioning is obtained as follow :

- 1) on arrival, two-month old rats are put in standard individual cages at a temperature of 20 °C and subjected to a 14/10 photoperiodical system.
- 2) for the first 5 days after their arrival, rats are given food and drink ad libitum. They are weighed at the same time each day.
- 3) for the following 5 days, they are put on a gradual fast so that they will loose 20 % of their initial weight.
- 4) for the next 5 days, at the same time each day, the animal is placed in a skinner box for 15 minutes. All behavioural signs are videotape recorded on the last day.
- 5) on the following day, group of conditioned animals are exposed to the toxic atmosphere for 30 minutes.
- 6) at the end of the exposure, animals are immediately returned to the skinner box to be individually filmed for 15 minutes.

Then "performances" of each animal (8 by test) are compared to those got the previous day before exposure with the help of videotape recordings. All the acts of each animal are registered, then acts are sorted and gathered according the specific area where there were done and the type of act in order to leave 9 fundamental descriptive signs in the behavioural grid. These signs are listed here under :

- acts near the cup (Coup)
- acts near the lever (Levi)
- acts in the round-trip area (Alre)
- acts in the elsewhere area (Aill)
- average immobilization duration (Dmoy)
- total immobilizations duration (Dtot)
- immobilizations number (Fimo)
- total activity (Acti)
- number of food pellets eaten (Boul)

These fundamental signs are also used to compare groups performances to each other.

### **Data treatment**

Because of the low number of animals by group and the relatively large inter-animal behavioural variations observed sometimes, statistically significant results were not obtained for all tested groups by direct classical statistical computation. In order to compare animal groups each other, we used a descriptive method of data treatment which is the principal components analysis (PCA). It gives a synthetic view of the information hold in the behavioural grid and the results must be considered as tendencies.



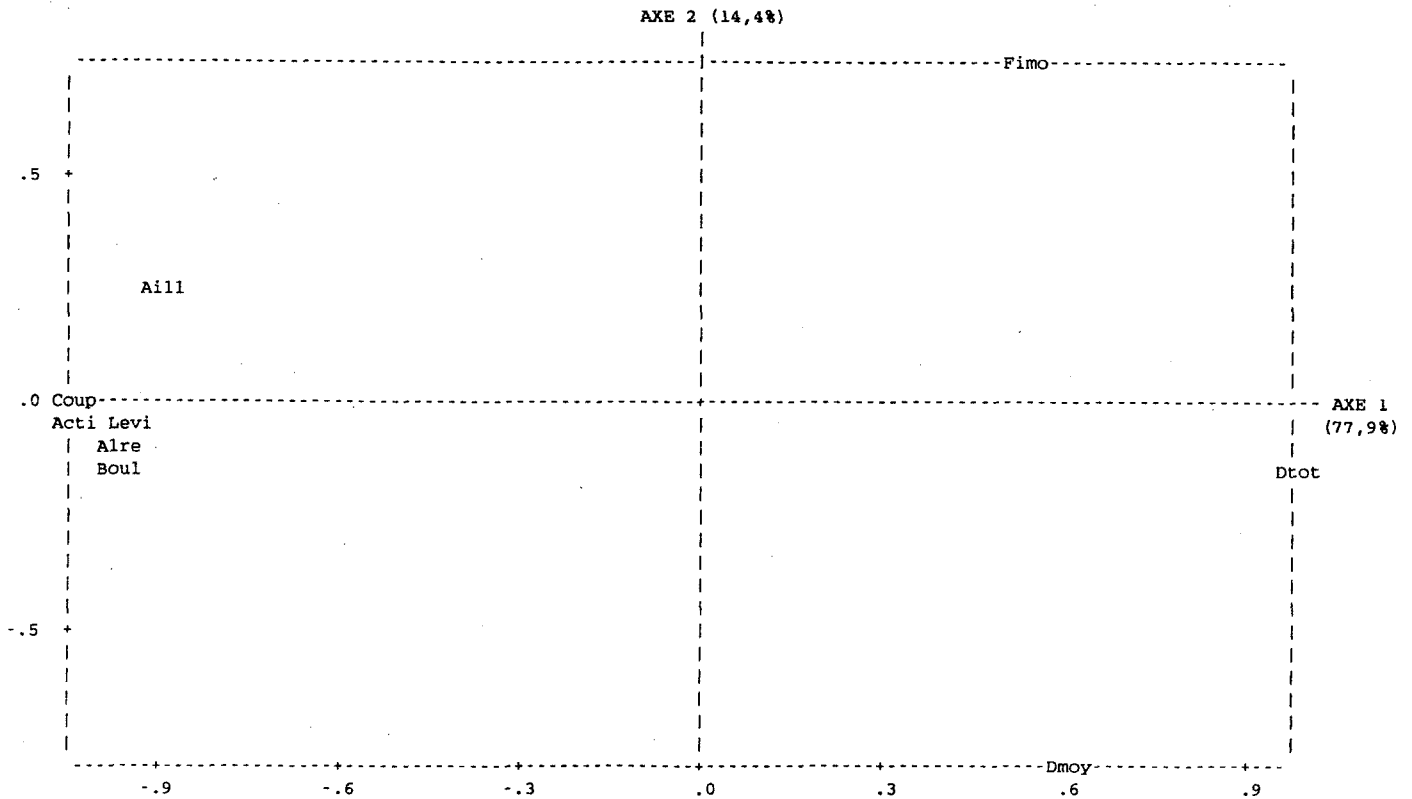
The PCA results are represented on 2 dimensions graphics because the 2 principal axis are sufficient to explain from 85 to 90 % of behavioural variations in all performed analysis.

Two types of graphics are issued from this statistical data treatment.

The first one presented on figure 4 is the result of the PCA of fundamental behavioural signs.

Axis 1 is characterized by the opposition between activity and conditioning signs (Acti, Levi, Coup, Alre, Boul) and the total immobilization duration (Dtot). It can be considered as the conditioning axis where high performance groups (or less incapacitated groups) are located on the left side of the axis, and the low performance groups (or more incapacitated groups) are located on the opposite side of the axis.

Axis 2 is explained by the opposition between the number of immobilizations (Fimo) and average immobilizations duration (Dmoy), or more clearly, high frequent immobilization but brief lasting groups and low frequent immobilisation but long lasting.



**Figure 4 : PCA of behavioural signs**

Through this figure, it seems that fundamental behavioural signs were correctly selected to reveal the incapacitation state and that they are strongly correlated following axis 1. Most of them are located on the high performance side of the figure and indicate thus that these signs are inter-correlated.

Axis 1 traduces the animals performance versus their initial conditioning and indicate the incapacitation level of exposed groups. The right end side location of groups is associated with animals presenting the highest level of incapacitation and thus exposed to the highest toxic atmosphere.

Axis 2 rather sorts the incapacitation level according to the type of animal response. Two exposed groups may have similar location according to axis 1 (so are equally incapacitated), but may be distinctly located according to axis 2. This may mean that their types of toxic impact induce different physiological responses (narcotic or irritant effect for example).

PCA of all exposed groups is presented on figure 5. Test mixtures are identified by their composition. A perfect virtual group (T) is included in the graphic in order to be a reference point. This point represents the location of a non-incapacitated group. The interpretation of this figure, and more precisely the mixtures arrangement according to axis 1 and 2 should give answers on interactive effects.

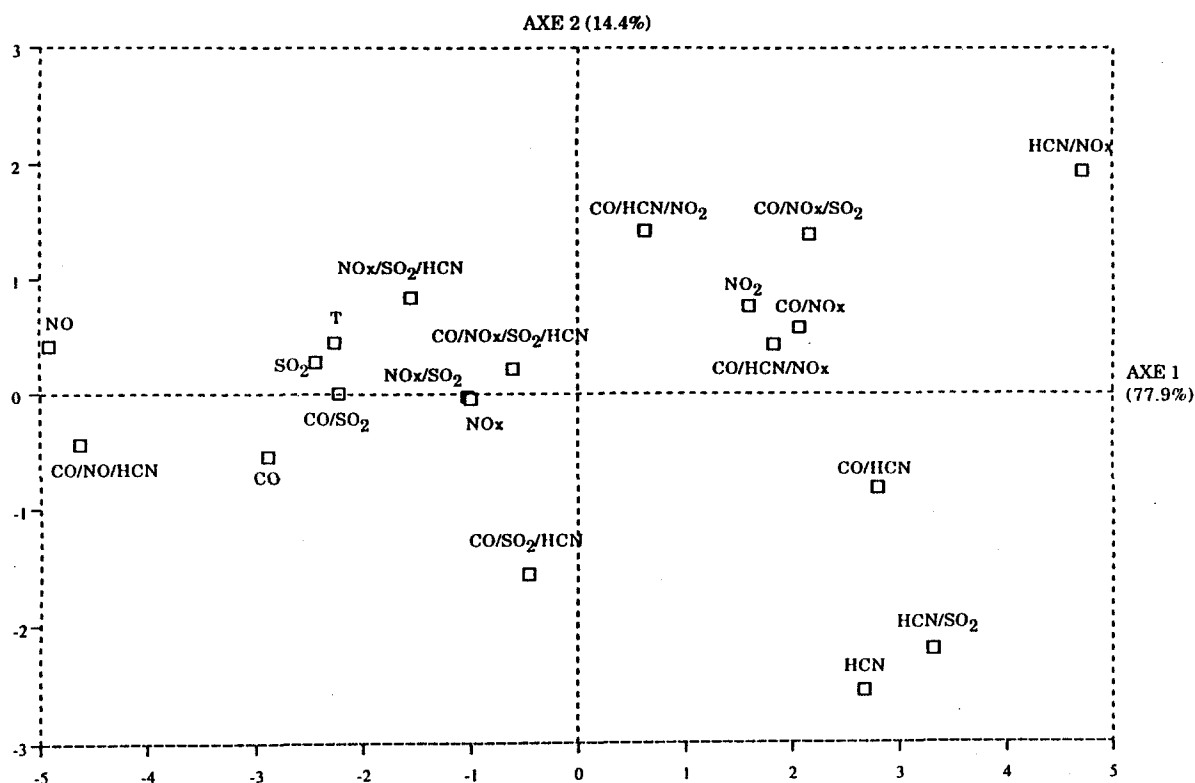


Figure 5 : PCA of exposed groups

## . MAIN RESULTS AND CONCLUSIONS

The groups arrangement covers all parts of the figure 5, and is thus indicating that gaseous toxic effects seem to be of various types and of different incapacitation levels.

Before concluding on gaseous interactions, we must recall that PCA results are only tendencies, and that they are linked to tested concentrations and to the rat for animal model.

## individual gases

Individual gases effects are presented on figure 6. It can be seen that :

- CO seems to produce very low toxic effect.
- HCN seems to be very incapacitating.
- NO tends to increase animals activity and can be considered as an exception among other gas toxic effects.
- NO<sub>2</sub> can be considered as a strong irritant gas. The incapacitating effect observed is in the same order of magnitude as HCN.
- SO<sub>2</sub> appears as a less powerful irritant gas than NO<sub>2</sub>, and induces a weak incapacitating effect in the same order of magnitude as CO.

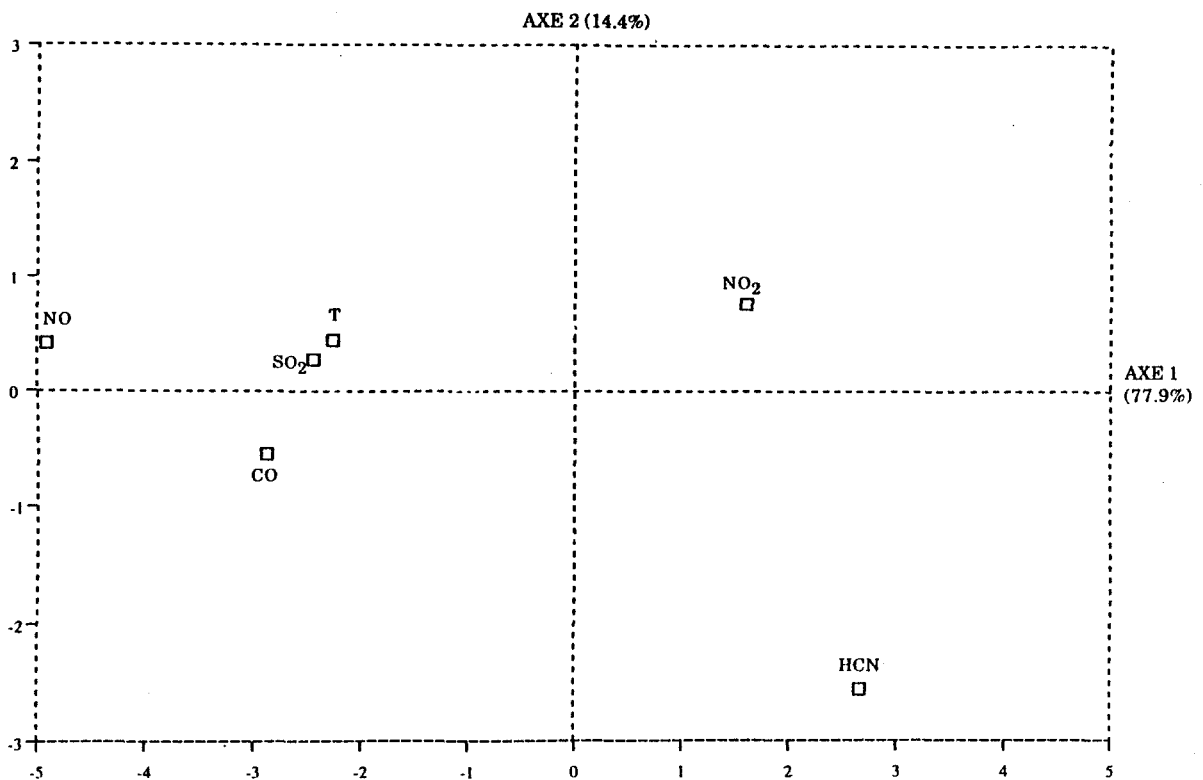


Figure 6 : individual gases effects

## binary mixtures

Binary mixtures arrangement is shown on figure 7. The respective toxic effects observed are function of interaction influences :

- mixed with an other gas, with the exception of NO, HCN remains a powerful incapacitating gas. The CO concentration level is too low to allow us to give a rule on the likely additivity of the narcotic effects of CO and HCN . The same comment can be applied to the HCN/SO<sub>2</sub> mixture but this time for a

supposed antagonistic effect. Moreover, clinical follow-up results seems to indicate that the interaction between HCN and SO<sub>2</sub> may be simple additivity.

- the mixture of NO and NO<sub>2</sub> to make (the so called) NO<sub>x</sub> induces a moderate effect, at intermediary level to the respective effects of NO and NO<sub>2</sub>. This may lead to suppose that an antagonistic effect appears with this mixture.

- the CO/SO<sub>2</sub> mixture reveals a very low toxicity, close to the control group (T), according to their respective individual toxic impact previously observed. A simple additivity interaction may be considered.

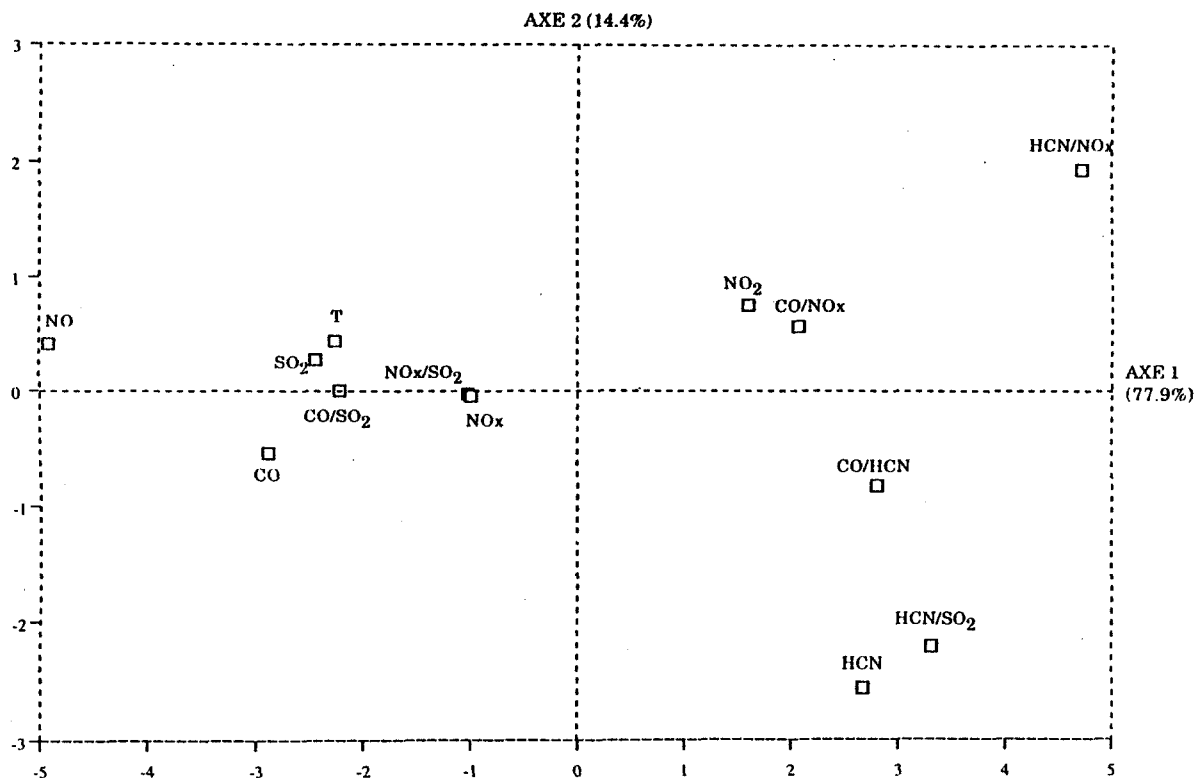


Figure 7 : binary mixtures

### complex mixtures

Complex mixtures arrangement is presented on figure 8.

When mixed to more than one other gas, HCN effect seems to be lowered. This is particularly clear in the NO/CO/HCN mixture where an antagonistic interaction between NO and HCN decreases the toxic impact of the mixture to the lowest incapacitation level.

Such an explanation may also be put forward in mixtures like CO/SO<sub>2</sub>/HCN and NO<sub>x</sub>/SO<sub>2</sub>/HCN. In both cases the HCN impact is reduced but the overall impact is more important than mixtures without HCN. However, mechanisms and natures

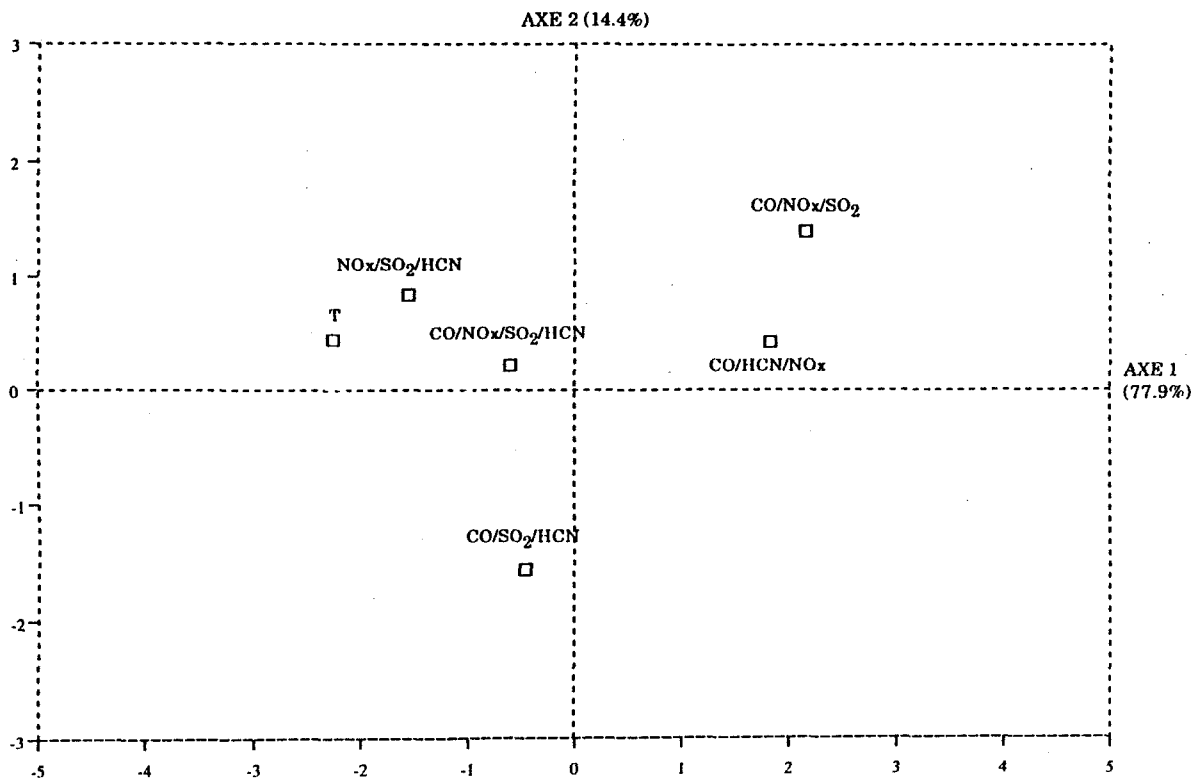
of these antagonistic interactions are not easy to identify because of the low number of performed tests in this study.

Most of the tested mixtures including NO<sub>x</sub> as a component seem to present a high immobilisation frequency (Fimo). This may be characteristic of the NO<sub>x</sub> presence in a mixture.

The CO/NO<sub>x</sub> mixture reveals an important toxic impact which could be the result of a synergetic effect.

The NO<sub>x</sub>/SO<sub>2</sub> mixture seems to reveal a simple additivity interaction.

Finally, the most complex mixtures (4 and 5 components), like CO/SO<sub>2</sub>/NO<sub>x</sub>/HCN or NO<sub>x</sub>/SO<sub>2</sub>/HCN, show the existence of interactions, generally antagonistical if the HCN exposure group is taken for reference, which are impossible to identify. In these cases, though mixtures are complex, revealed toxic impacts are in the same order of magnitude as an individual moderate toxicant exposure.



**Figure 8 : complex mixtures effects**

### **conclusion**

The results of this study are in relatively good agreement with specialized literature on the subject of gaseous toxic interactions.

However, examination of tendencies show that there are no general rules that can be deduced from them in order to define toxic interactions appearing in the three

possible types of mixtures :

- narcotic + narcotic
- irritant + irritant
- irritant + narcotic

Global effects observed are issued from numerous and various interactions which seem to depend on the nature of the gases contained in the mixtures.

General rules on interactive phenomena cannot be drawn-out with the low number of performed tests in this study. So this research need to be continued.

This paper shows that the behavioural method developped in the study is adapted to the defined objectives, and that data treatment results are relatively easy to translate into terms of toxic impact and likely associated interactions.

This study need to be continued with new parameters such as concentration levels of CO and SO<sub>2</sub> increased to reach about 50 % of their respective LC<sub>50</sub> with the aim to reveal more easily interactive effects, and with variations of concentration of each gas to approach these influence on interaction levels.

It must also be reminded that clinical follow-up results (not shown here) are quite well correlated with behavioural results, and that CO and CN blood analysis are quite well correlated too. These complementary measurements appear necessary to confirm the behavioural results.

The biochemical investigations must also be kept in order to identify mechanisms involved in interactive phenomena.

### **Acknowledgements**

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