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# Potential of the Trad-MCN assay applied with inflorescences of *Tradescantia pallida* 'Purpurea' for evaluating air contamination by naphthalene

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**Abstract**

The aims of this study were to determine clastogenic responses of *Tradescantia pallida* cv. Purpurea to naphthalene (NAPH) by means of the bioassay Trad-MCN with inflorescences of *T. pallida* cv. Purpurea and to verify if this assay might be an indicator of the potential risk imposed in a workplace, where solid insecticide containing NAPH is usually applied. The clastogenic potential of NAPH was assessed by using static and dynamic experimental systems. In both systems, increased micronucleus frequencies were observed in inflorescences submitted to increasing concentrations of solid or gaseous NAPH. The evident clastogenicity verified in inflorescences exposed experimentally to 25–50 mg $m^{-3}$ of NAPH during 6 h points to a narrow threshold of plant sensitivity, indicating risks under lower NAPH levels than the standards established by OSHA and therefore revealing its suitability for biomonitoring purposes. However, the clastogenic risk should be carefully investigated by other monitoring methods if human health is taken into consideration.


*Keywords:* Clastogenicity; Herbarium; Naphthalene; *Tradescantia pallida* 'Purpurea'; Trad-MCN

## 1. Introduction

Naphthalene (NAPH, $C_{10}H_8$; CAS number 91-20-3) is a compound of the polycyclic aromatic hydrocarbon (PAH) class, characterized by two or more condensed aromatic rings. These substances and their by-products have been present in different environments. They come from different sources and are found both outdoors and indoors. It is the most volatile compound among those of the PAH class and it pollutes the environment by incomplete combustion processes from industrial, domestic, and natural sources, for example, fossil fuels burning and forest fires (Preuss et al., 2003). Photocopy machines, cigarette smoke and a wide range of chemicals used for cleaning and sanitizing environments are the major emission sources of indoor hydrocarbons (Pereira Netto et al., 2000; Schreiner, 2003; Preuss et al., 2003). Insecticides widely used in houses and working environments are also a source of indoor contamination, especially NAPH.

NAPH causes many health problems to human beings, e.g. eye irritation, headache, malaise, nausea, abdominal pain, dermatitis and sweating, as well as damage to organs such as liver, kidneys, and skin (OSHA, 2002). Due to this, the Occupational Safety and Health Administration (OSHA) established a threshold limit value (TLV) of 50 mg $m^{-3}$ for environmental and occupational NAPH exposure.

The clastogenicity of NAPH to other organisms is reported by many authors (Pereira Netto et al., 2000; Preuss et al., 2003; Schreiner, 2003; Aina et al., 2006). According to Schreiner (2003), NAPH induces genotoxic effects in a number of *in vitro* assays with mammalian cells. Neoplastic and non-neoplastic lesions in the nasal mucosa were detected in rodents after 2 years of exposure to 10, 30,

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and 60 ppm of NAPH vapors (Long et al., 2003). Aina et al. (2006) also observed significant changes in the sequence of DNA in roots and shoots of *Trifolium repens*.

The carcinogenic effects of the PAHs in human beings originate from the breakage of DNA structure induced by their sub-products, such as diol-epoxides. By contrast, as naphthalene diol-epoxide can be rapidly hydrolyzed in non-genotoxic compounds, it is believed that the effects on DNA caused by NAPH are provoked by quinones arising from NAPH metabolism (Preuss et al., 2003). Anyway, it is reasonable to suppose that genotoxic risks imposed by NAPH in the environment may be adequately monitored by bioassays that involve measurements on chromosomal alterations.

The Trad-MCN test applied with inflorescences of clones of *Tradescantia* (Commelinaceae) is frequently used to monitor genotoxic risks (Ma, 1981). It consists of estimating frequency of micronuclei (MCN), resulting from chromosome breaks after exposure to genotoxic agents, in pollen mother cells in the early tetrad phase. Additionally, Batalha et al. (1999) and Suyama et al. (2002) demonstrated under experimental conditions that this assay can be satisfactorily carried out with inflorescences of *Tradescantia pallida* cv. Purpurea. Miyazato (1999) and Alves et al. (2003) also showed that this cultivar assessed effectively the clastogenic risks in workplaces. Thus, we aimed: (1) to determine the intensity of clastogenic responses of *T. pallida* cv. Purpurea to NAPH, by means of the establishment of dose–response relations under experimental conditions; (2) to verify if this assay developed with inflorescences of *T. pallida* 'Purpurea' is appropriate to give an indication of the potential risk imposed in a workplace where solid insecticide containing NAPH is usually applied. In both cases, biological and analytical methods were comparatively used.

## 2. Materials and methods

The relations between doses of NAPH in the air and frequency of MCN in pollen mother cells of inflorescences of *T. pallida* 'Purpurea' were determined by means of two experimental systems. In the first one (static system), the cuttings were exposed for 6 h to increasing amounts of solid NAPH insecticide (2.1, 4.2, and 8.3 mg $m^{-3}$) in a glass chamber without a flux of air. These amounts were established based on the amount of product used in each steel cabinet of the Herbarium of São Paulo State. After that, other inflorescences were exposed to known doses of solid NAPH (1.0, 1.5, and 2.0 g), which produced 25, 110, and 220 mg $m^{-3}$ of gaseous NAPH, respectively, in a dynamic chamber system at a flow rate of 5 L $h^{-1}$. In both experiments, the control treatment was simulated by exposing a group of cuttings to the same system conditions but without NAPH.

The indoor exposures were performed at three sites of the mentioned Herbarium, where solid NAPH has been applied for many years for protection of vascular plant exsiccates against insect attacks. The criterion for exposure site selection was the distance from the area directly exposed to NAPH (collection area) to adjacent areas (hallway and office). The collection area is completely isolated by wood walls and has no ventilation at all. The plant collection is stored in steel cabinets, each one containing about 2 mg $m^{-3}$ of solid NAPH, which are replaced twice a year. All compartments of the Herbarium have no air acclimatization, so that the indoor temperature changes according to the season of the year. Due to this, cutting exposures at the mentioned sites of the Herbarium were performed for 6 h in 1 day in the winter, in the spring and in the summer times. Two other sets of inflorescences were simultaneously maintained in a greenhouse under filtered air during each exposure, simulating the negative and positive controls (nutrient solution and 10 ppm formaldehyde solution, respectively).

All the experiments were performed with inflorescences taken from plants of *T. pallida* 'Purpurea' cultivated by vegetative propagation in a standardized substrate (bark of *Pinus* and vermiculite 3:1) in plastic pots, and maintained in the greenhouse under filtered air, free from NAPH and other potentially hazardous chemicals. They were immersed in nutrient solution prepared according to the Epstein (1975) and continuously aired with a compression pump. The experiments started with 20 inflorescences per treatment, always only after keeping them for 24 h in such situation for adaptation. After exposures, the inflorescences were taken again to the greenhouse under filtered air, where they remained for 24 h a period that was named as recovery time by Ma (1981). After that, they were fixed in ethanol/glacial acetic acid solution 95% (1:3) for 24 h and stored in ethanol 95% for estimating the frequency of MCN. Slide preparation and micronucleus scoring in early tetrads were performed throughout the experiments according to the protocol proposed by Ma (1981). The results were expressed as the number of micronuclei per 100 early tetrads.

Gaseous NAPH was collected during the experiments developed at the dynamic chamber system and at three exposure sites of the Herbarium, during the summer experiment, with a portable pump connected to stainless steel tubes containing 100 mg of Chromosorb 106 at a flow rate of 0.8 L $min^{-1}$ for 15 min. A flow meter was used to measure the total volume of air sampled. After collection, the NAPH was extracted from the adsorbent with 1 mL of dichloromethane under mechanical shaking, for 60 min, at room temperature (28 °C). The extracts were analyzed by gas chromatograph equipped with mass spectrometer GC/MS (Agilent), according to the method proposed by NIOSHI (1994). The species was identified by a comparison of standard retention times and confirmed by MS analysis.

One-way ANOVA and the Newman–Keuls and Bonferroni multiple comparison tests (both for $P<0.05$) were used to identify differences among the results.

## 3. Results

In the static system, significant increases in the micronucleus frequency were observed after exposure of inflorescences to all solid NAPH treatments, compared to the results from the control situation, but 4.2 or 8.3 mg $m^{-3}$

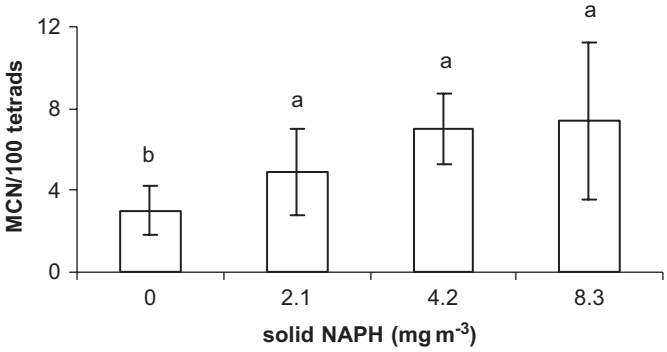


Fig. 1. Mean values and corresponding standard errors of micronucleus frequencies in cuttings of *Tradescantia pallida* 'Purpurea' after exposure to different levels of solid naphthalene (NAPH) in the fumigation static chamber. Mean values indicated by distinct letters differ significantly from each other ($P<0.05$).