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CASE REPORT

Validation of a new adhesive coating solution: comparative study of carbon tetrachloride and diethyl ether

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Abstract Allergy is a public health problem that affects a significant part of the population. In Europe, pollen allergy is the most common form of seasonal allergic respiratory disease, and in Western Europe, nearly 20 % of the population currently suffers from an allergy to pollen (Thibaudon et al. in Rev Mal Respir 30(6):463–479, 2013). Epidemiological studies conducted in Europe and other parts of the world in the late twentieth century show that allergic diseases (i.e. rhinoconjunctivitis, asthma, eczema) have almost tripled in the last 30 years. In order to measure the exposure of the European population to allergenic pollen, networks of pollen monitoring sites have been set up using standardised techniques. Hirst-type volumetric spore traps are routinely used to capture airborne pollen. The biological particles are impacted onto a transparent strip that is coated with an adhesive. However, due to regulations, some products are prohibited and are no longer produced, as is the case of carbon tetrachloride (CCl_4) commonly used as an adhesive. In this study, we examined the impaction efficiency of CCl_4 and diethyl ether ($(C_2H_5)_2O$) over several weeks. The results show that there is no significant difference between the two silicone solvents and that diethyl ether may replace CCl_4 for coating the tape.

Keywords Aerobiology · Airborne pollen monitoring · Biological particles · Quality control · Pollen trap · Pollen counts

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1 Introduction

A variety of airborne biological particles (e.g. some pollen grains and fungal spores) can have an impact on human health. In Europe, approximately 18–20 % of people suffer from allergy due to pollen and/or fungal spores and allergic diseases (i.e. rhinoconjunctivitis, asthma, eczema) have almost tripled in the last 30 years (Thibaudon et al. 2013). In some European Member States (for instance, in France and in Spain), pollen grains are considered to be an air pollutant, along with other particles suspended in the air (PM10, PM2.5). With this in mind, the European Aerobiology Society (EAS) has set up a number of different working groups, some of which are working towards the standardisation, Quality Assurance and Quality Control of data produced by airborne pollen monitoring networks. This work is carried out in collaboration with the International Association for Aerobiology

(IAA) and the European Aeroallergen Network (EAN). The EAS is also involved with different tasks regarding education, research and dissemination of information.

All members involved in the EAN use a standardised method (Jäger et al. 1995; Galán et al. 2014), i.e. using the Hirst-type volumetric spore trap (Hirst 1952) and expressing results as a daily average of pollen grains or fungal spores per cubic metre of air (pollen grains/m^3). The Hirst-type trap works by the impaction of airborne biological particles onto a transparent plastic strip (for weekly monitoring) or glass slide (for daily monitoring) (Thibaudon et al. 2013). In both cases, an adhesive substance is used for capturing airborne particles present in the air samples.

At present, two transparent coating products (solubilised in specific solvents) are used: petroleum jelly (Vaseline®) or silicone. Vaseline® solubilised with toluene can be used, but its capacity is limited because it becomes liquid at 40 °C (Galán and Dominguez-Vilches 1997). Silicone, initially proposed by Hogan (1971), maintains a constant viscosity from 0 to 200 °C, and it is currently solubilised with carbon tetrachloride (CCl_4). However, this solvent has recently been prohibited by the Registration Evaluation Authorisation of Chemicals (REACH) protocol because it poses a danger to human health and environment, and its carcinogenic properties (Manibusan et al. 2007). Silicone fluid can continue to be used as a capture medium, but an alternative solvent must be found to replace CCl_4. The main goal of this study, therefore, was to evaluate the capturing efficiency of a new coating solution diethyl ether ($(C_2H_5)_2O$) in comparison with the reference medium (CCl_4).

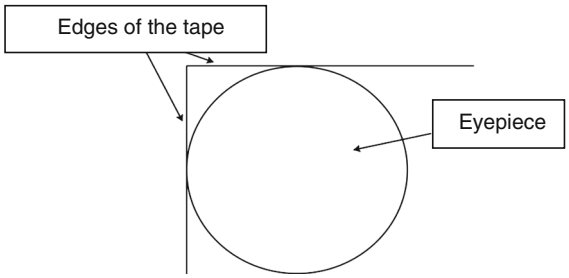

Fig. 1 Reading microscope slides methodology 1: location of the microscope slide on the stage

2 Materials and methods

This study has been carried out using a Hirst-type volumetric spore trap (Hirst 1952) located at two sites, Córdoba (Spain) and Brussieu (France), following the same protocol. The experiment was carried out by coating the tape on the drum with silicone adhesive containing two different solvents: (A) one half of the tape was coated in a solution containing diethyl ether; (B) and the other half was coated in a solution containing CCl_4.

In Spain, sampling was carried out over an 8-week period. This represents 56 days from 6th September to 31st October, 2013. After this date, the amounts of

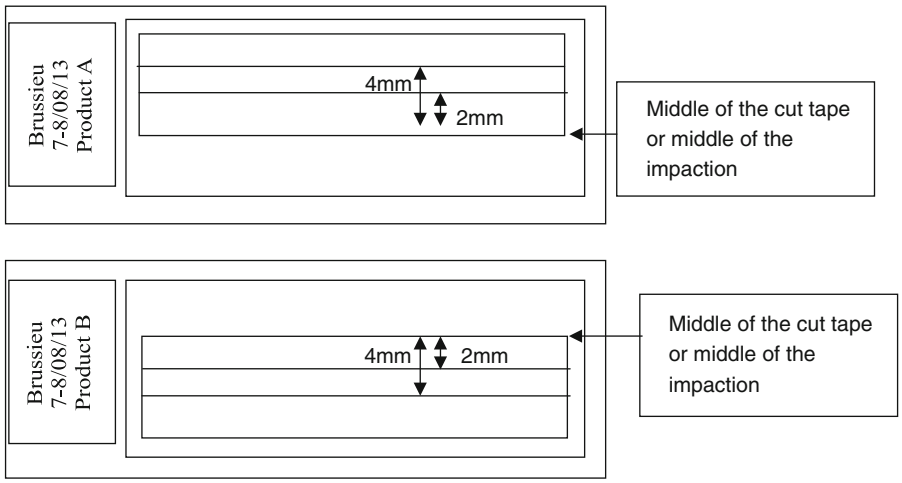

Fig. 2 Reading microscope slides methodology 2: reading mode on the microscope

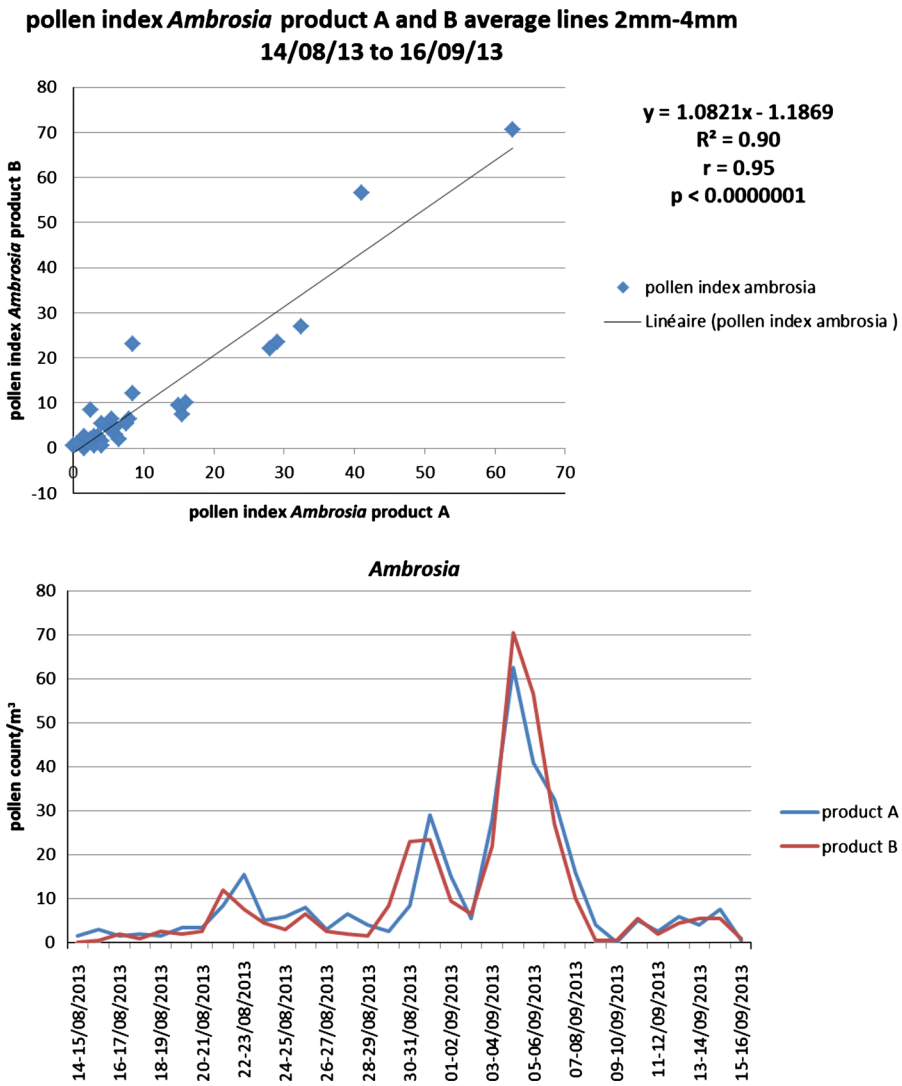

Fig. 3 Pollen index of *Ambrosia* in Brussieu (France), 14/08/2013 to 16/09/2013. Comparison between the products A and B. Average lines 2–4 mm

airborne pollen were low, because it was autumn, and there were not enough data for statistical analysis.

In France, samples were collected over a 6-week period. This represents 43 days, from 5 August to 16 September, 2013. However, due to the paucity of pollen during the first day of August, only the 34-day period from 14th August to 16th September was included in the statistical study.

Technical staff examined two different pollen types for this study, choosing those most representative in the air with higher concentrations during the studied period, and also the Total Pollen Count (TPC).

- Cupressaceae, Amaranthaceae and TPC for Spain;
- *Ambrosia*, Urticaceae and TPC for France.

The methodology used for preparing the tapes, drums and slides is presented below.

2.1 Tape preparation

The weekly transparent tapes for sampling with drum were cut lengthwise:

These half-tapes were fixed onto the drum and coated with different silicon solvents, (A) one of them with the new diethyl ether solution; (B) and another with the former CCl_4 solution.

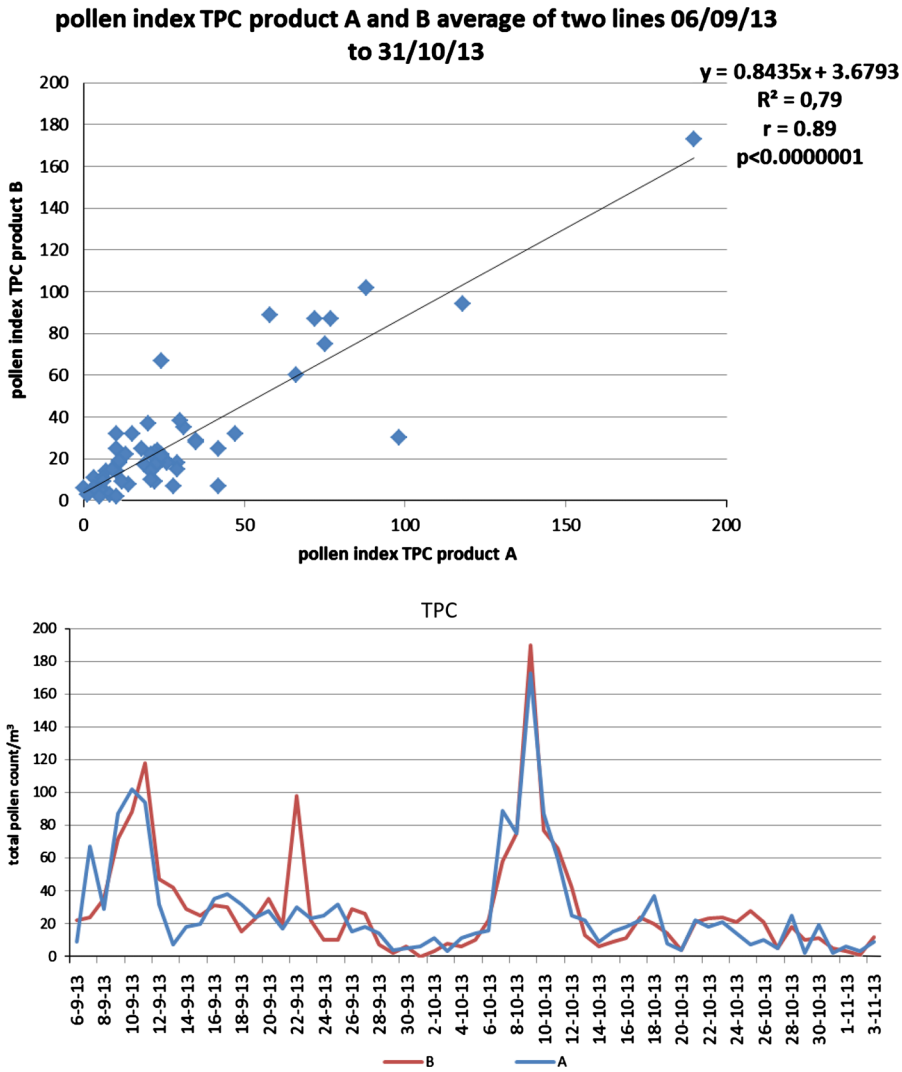

Fig. 4 Pollen index TPC in Córdoba (Spain), 06/09/2013 to 31/10/2013. Comparison between the products A and B. Average lines 2–4 mm

2.2 Drum preparation

In order to avoid introducing some level of bias into the validation, the same drum has been used throughout the experiment with the coated half-tapes.

The beginning of the first half-tape coated with solution A was placed on the right side of the sticky tape, and the beginning of the first half-tape coated with solution B was placed on the left side of the sticky tape with cleaned pliers; then the tape was placed around the drum.

The position of the two half sampling tapes from right to left position has been reversed every next preparation of the drum (i.e. every week).

2.3 Slide preparation

Both weekly coating tape with solution A and B were divided on seven 24-h segments. Fourteen slides *per* week were obtained for analysis under optical microscopy.

Slides were labelled (on the left) with the date and the solution used.

2.4 Slide reading

A slide was put on the microscope stage (label on the left). It is known that with the Hirst-type trap, there is a loss of pollen grains from the centre to the outside of

Table 1 Relationship between the pollen indices obtained, respectively, with the adhesive coating solution A (containing diethyl ether as silicon solvent) and the adhesive coating solution B (containing CCl_4 as silicon solvent): the main statistics values

	Linear correlation			Wilcoxon's test		
	R^2	r	p value	Z (based on positive ranks)	Asymp. Sig. (2-tailed)	p
France ($n = 34$ days)						
Urticaceae A versus Urticaceae B	0.92	0.96	$p < 0.0000001$	−0.638	0.523	$p > 0.05$
Ambrosia A versus Ambrosia B	0.90	0.95	$p < 0.0000001$	−1.532	0.126	$p > 0.05$
TPC A versus TPC B	0.83	0.91	$p < 0.0000001$	−1.010	0.312	$p > 0.05$
Spain ($n = 56$ days)						
Cupressaceae. A versus Cupressaceae B	0.96	0.98	$p < 0.0000001$	−0.566	0.572	$p > 0.05$
Amaranthaceae A versus Amaranthaceae B	0.71	0.84	$p < 0.0000001$	−0.524	0.600	$p > 0.05$
TPC A versus TPC B	0.79	0.89	$p < 0.0000001$	−0.200	0.842	$p > 0.05$

the tape (Tormo-Molina et al. 1996). Therefore, the microscope was initially positioned on the side of the tape that corresponds to the area where most particles were impacted. The microscope was placed in the corner of the tape, so that the edges of the field of view lined up with the edges of the tape (Fig. 1). Two horizontal lines per slide were analysed: 2 and 4 mm away from the centre of impaction (Fig. 2).

2.5 Statistical analysis

The relationship between the two products has been presented graphically by scatter plot. The strength of the relation has been expressed by the coefficient of determination (R^2), which was calculated by using linear regression trends.

Shapiro–Wilk's test has been applied to test data for normality. The data were not normally distributed and so nonparametric statistics were used. The Wilcoxon test has been applied to pollen data recorded on the two adhesives to test whether there were significant relationships between the two.

The statistical analysis of the pollen counts detected with the two solutions was performed with both Excel® (in order to create scatter diagrams and calculate Bravais–Pearson correlation coefficients) and the Statistical Package for the Social Sciences SPSS® (for applying the Wilcoxon signed-rank test). An average of the two horizontal lines (2 and 4 mm) *per* adhesive coating solution was performed for each result from both, France and Spain.

3 Results and discussion

Due to the fact that under the REACH regulations CCl_4 will no longer be produced, it is important to find a new solvent for the silicon-coating solution. Diethyl ether, also simply known as ether or ethoxyethane, is an organic compound that is a highly volatile flammable liquid, routinely used as a laboratory solvent. It has only a low acute toxicity, and there is no evidence of carcinogenic or teratogenic effects attributable to its use (Pohanish 2012).

Analysis has shown that silicone adhesives containing either diethyl ether or CCl_4 solvent produced similar results for capturing airborne pollen. The scatter plots provide visual representations of the relationship between the pollen counts produced using the two adhesive coating solutions (Figs. 3, 4). The results have shown a positive linear correlation between the two variables for all pollen types, and also for the TPC in both France and Spain, with coefficients of determination from 0.84 to 0.98 and $p < 0.0000001$ (Table 1). In addition, the Wilcoxon test for comparing nonparametric paired data have shown that there were no significant differences for counts of selected pollen types and TPC at the two

sites in France and Spain ($p > 0.05$) (Table 1). It has also been observed that the transition from one adhesive coating solution to another does not alter the capture efficiency.

4 Conclusion

These results have shown strong correlation between silicone fluid produced using the two solvents CCl_4 and diethyl ether. Diethyl ether solvent can therefore be used as a substitute for the CCl_4 solvent, which has recently been outlawed by the REACH regulations, without alteration to the capture efficiency of the sampler. This study validates the use of a new adhesive coating solution, and so the EAS and IAA can recommend its use in the future.

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