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Assessment of chronic effects of 1,3,5-trimethyl-benzene (mesitylene) on plants

Th.A. Dueck & C.J. van Dijk

Report 203

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Plant Research International B.V.

Address : Droevendaalsesteeg 1, Wageningen, The Netherlands
: P.O. Box 16, 6700 AA Wageningen, The Netherlands
Tel. : +31 317 48 60 01
Fax : +31 317 41 80 94
E-mail : info.pri@wur.nl
Internet : www.pri.wur.nl

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Summary

Recommendations in the current risk assessment of gasolines and naphthas state that the vapour phase phytotoxicity of certain blocks of hydrocarbon to plants requires investigation. A number of hydrocarbons representing these blocks were chosen to be tested, among others, mesitylene. A fumigation experiment was performed in which a number of plant species representing the European flora was exposed to a range of mesitylene concentrations. Pressurised nitrogen was bubbled through warmed liquid mesitylene to bring it into the gas phase. It was then injected into the air-stream entering the plant fumigation chambers at constant concentrations for a period of up to 12 weeks. The mean measured concentrations were 0.12, 1.22, 3.5, 10.6, 12.5 and 29 ppmV mesitylene. The main objective of the study was to quantify the effects of mesitylene on plants and derive no-observed-effect concentrations (NOECs) for each plant species. However, even at the highest concentration of mesitylene used in this study no significant effects on the measured endpoints were found, and thus NOECs could not be calculated for any of the plant species tested. Subsequently, an analysis of variance was performed for significant (negative) trends in response to increasing concentrations of mesitylene. No trends were observed, which leads us to the conclusion that mesitylene up to concentrations of 30 ppmVV does not negatively affect plants.

1 Introduction and aims of the study

Recommendations in the current risk assessment of gasolines and naphthas state that the vapour phase toxicity of certain hydrocarbon blocks to plants requires investigation. In order to minimise extended testing of the many hydrocarbons of relevance, this project proposes to test a number of representative hydrocarbons and via quantitative structure relationships, estimate effect characterizations of untested hydrocarbons.

Mesitylene was chosen as the second of these hydrocarbons to be tested. The vapour phase exposure test was performed in the spring of 2007. It was based on an earlier test protocol for DBP (Dueck *et al.*, 2003) and complimented by experience with other air pollutants and air pollution fumigation studies, including undecane (Dueck & Van Dijk, 2008) at these facilities.

The objective of the study was to quantify the effects of mesitylene vapour on plants and derive, if possible, no-observed-effect-concentrations (NOECs) for each plant species.

2 Materials and Methods

The relevant methodology for the vapour phase testing of 1,3,5-trimethylbenzene (mesitylene) will be briefly stated below.

2.1 Test substance

The test substance 1,3,5-trimethylbenzene, further referred to as mesitylene is an aromatic hydrocarbon with three methyl substituents attached to the benzene ring. It is a major urban volatile organic compound and its chemical formula is $CH_3(CH_2)_9CH_3$. It was obtained from Fisher Emergo, purity 99%. The technical details are:

Chemical identity:	1,3,5-trimethylbenzene
Chemical formula:	C_9H_{12}
Molecular weight:	120.9 g mol^{-1}
Appearance:	clear fluid
CAS-No:	108-67-8
Melting point:	-44.8°C
Boiling point:	164.7°C
Vapour pressure (25°C):	1.17 mm Hg
log Koa	4.41
log Kow	3.36

2.2 Exposure facilities and treatment applications

The fumigation experiment was performed in six closed fumigation chambers located in a climate room (15 m^2) at Plant Research International B.V., Wageningen in The Netherlands. The fumigations consisted of a control and five exposure treatments. The airtight fumigation chambers were made from hardened glass set in an aluminium framework (0.85 x 1.0 x 0.9 m; 765 litre), with a stainless steel floor. Tubing for outgoing air was made of teflon. Both incoming and outgoing air streams were passed through an activated charcoal filter. Incoming air entered each chamber under a perforated floor at an air exchange rate of 0.5 m^3 min^{-1} and was continuously recirculated at a rate of 4 m^3 min^{-1} before being blown off into the outdoor air. A lower atmospheric pressure was maintained in the fumigation chambers to avoid contamination in the climate chamber. Temperature, air humidity and light intensity were recorded inside the chambers. Wind speed and turbulence in the fumigation chambers was maintained at 0.5 m s^{-1} with additional ventilators to ensure gas exchange.

Pressurised nitrogen, controlled by thermal mass-flow controllers, was led through commercially available liquid mesitylene in a vaporiser unit, which was maintained at 23°C. The saturated air stream was mixed with a small amount of ambient air and passed through stainless steel tubing, insulated and warmed to 30°C to avoid condensation in the tubing, before being injected into the fumigation chamber under the perforated floor. There it was mixed into the recirculating air (4 m^3 min^{-1}) in the chamber. Thus, controlled concentrations of atmospheric mesitylene were produced and injected into the air for 24 h day^{-1}.

2.3 Fumigation treatments

Prior to the actual test, a range-finding experiment was performed from January 15th to March 9th 2007 in order to estimate mesitylene concentrations that were non-lethal, but were still considered to result in undesired effects on plant growth and functioning. Five plant species (*Phaseolus vulgaris, Trifolium repens, Solanum nigrum, Holcus lanatus* and *Plantago lanceolata* were exposed to mesitylene. Plants were taken from the greenhouse and placed in

the fumigation chambers for one day to acclimatize. An initial mesitylene concentration range was chosen (control, 0.1, 0.3, 1, 3 and 9 ppmV) to which plants were exposed for several days. The concentration in the highest treatment was then increased until a visual, qualitative effect was observed at 15 ppmV.

Based on the results of the range-finding tests the fumigation system was set up for six target exposure levels: control, 1, 3, 9, 15 and 27 ppmV[1] mesitylene. The highest possible mesitylene concentration in air that could be generated was ca. 30 ppmV. The experimental exposure period was chosen to continue for a period of 12 weeks, or less for a particular species if it flowered and produced seed earlier.

2.4 Measurement of mesitylene in the test chambers

Sampling. Air samples were taken with an automated gas sampling device. The samples were taken with a flow of 10 ml min^{-1}, controlled with programmable mass flow controllers. Teflon tubing from the chamber to the trapping tube was flushed for 5 min. prior to trapping . Samples were pumped through tubes filled with 200 mg Tenax TA for different time intervals (10-20 min) to maintain the concentration within the range of the calibration curve. Each fumigation chamber was sampled twice weekly in duplicate.

Sample Analysis. Analysis was performed by thermal desorption-GC-MS. Samples were desorbed via an Ultra autosampler (Markes International Ltd, UK) by heating the tube for 4 minutes to 250 °C with a helium flow of 30 ml min^{-1}, a split flow of 46 ml min^{-1} and focussed on a unity injector (Markes International Ltd, UK) on a trap containing a multibedsorbent at 10 °C. Compounds were injected into the capillary column (RTX-MS, 30 m, 0.25 µm id, 1.4 µm df) by rapidly heating the trap to 250 °C at 12 °C s^{-1}, using a column flow of 1 ml min^{-1} and a split flow of 49 ml min^{-1}.

The GC (trace GC ultra, Interscience, the Netherlands) was programmed at 60 °C for 2.5 minutes, then to 280 °C with a ramp of 20 °C $minute^{-1}$ followed by 1.5 minutes at 280 °C. Quantification was performed by spiking blank Tenax tubes with different quantities of mesitylene to acquire a calibration curve. The tubes were analysed in the same way as the sampled tubes. Samples were measured on a DSQ mass spectrometer (Interscience, The Netherlands) in full scan mode (mass 35-300) at 952 Amu sec^{-1}. Mass 120 was used for quantification.

2.5 Choice of plant species and endpoints

The plant species chosen for the experiment were representative of the European flora and included plant species representative for crops, trees and natural vegetation (Table 1).

Plant growth is considered to be the most important response (endpoint) to air pollutant exposures in relation to consequences at the population level. For annual species, generative reproduction is also important and this developmental phase is usually accomplished within 8-12 weeks. Perennial species will not always have flowered within that time period, but will have realized the largest proportion of vegetative growth.

During the fumigation period, daily observations were made with respect to plant appearance. Every 7-10 days all plants were taken out of the fumigation chambers and scored for injury and general appearance, i.e. chlorose, necrose, leaf morphology, number of flowers. Furthermore, the relative amount of chlorophyll was estimated by measuring light transmission through the leaves with a handheld meter (Minolta SPAD 50). Individual representative leaves on each plant were chosen for these measurements.

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1 1 ppmV mesitylene = 4.91 mg m^{-3} at 25°C and 760 mm Hg

Table 1. Plant species selected for the hydrocarbon toxicity test and relevant effect-parameters.

	Plant species	Special characteristic	Relevant effect-parameters
Crops	*Phaseolus vulgaris* (bean)	Nitrogen fixing dicot	shoot and root biomass, pod weight, number of pods
	Brassica campestris (cabbage)	Waxy leaves	shoot and root biomass
Trees	*Picea abies* (Norway spruce)	Evergreen	visual injury, biomass current year branches
Natural Vegetation	*Trifolium repens* (white clover)	Nitrogen fixer	shoot and root biomass, number of flowers
	Solanum nigrum (black nightshade)	Ruderal species	shoot and root biomass, number of berries
	Holcus lanatus (common velvet grass)	Common grass	shoot and root biomass, number of tillers
	Plantago lanceolata (narrowleaf plantain)	Ruderal species	shoot and root biomass, height and number of flowers

At harvest, the relevant effect-parameters mentioned in Table 1, shoot and root biomass, vegetative and generative (flower and seed) production, were taken and dried at 95°C to a constant weight and weighed.

2.6 Statistical analysis

The experimental design entailed a large number of treatments without replication in order to characterize dose-response relationships, and ultimately, NOECs. This implied using a regression approach as the basis for data analysis rather than ANOVA to test for differences between treatments.

Data for the response variables were averaged for each exposure level (fumigation chamber) prior to analysis. Treatment means for each species and response parameter were subjected to regression analyses with a logistic model (Genstat, 1993) to derive response curves. The best fit from a non-linear regression approach was used and applied in the calculation of regression equations using the formula:

$$y = C/\{1 + \exp[-B(\ln(x) - \ln(M)]\}$$

Where C is the calculated response at x=0 and x is the mean pollutant concentration (ppmV) during the exposure period relevant to each species. After estimating M (the pollutant concentration at which biomass is 50% of the control) and B (scale parameter), the Effective Concentrations at 10% (EC10) below C were to be calculated. Following this, No Observable Effect Concentrations (NOECs) were to be calculated for each species according to the formula:

$$NOEC = M - \{\ln(C/yc-1)\}/B$$

where yc is the lower limit of the 95% confidence limit of the asymptote (C). The NOECs and EC10 were to be calculated using the module Fitnonlinear of the statistical package Genstat.

However, due to the lack of treatment effects, the analysis did not produce the necessary parameters indicated above, and thus, no significant dose-response relationship could be identified.

We then used a standard variation analysis (Genstat, 1993) to test whether or not a trend was present over the range of treatment concentrations. The trend uses the experimental data to estimate if the regression line differs significantly from zero. The presence of a trend however, does not necessarily indicate an adverse treatment related effect.

3 Results

3.1 Range finding test

Five plant species (*Phaseolus vulgaris, Trifolium repens, Solanum nigrum, Holcus lanatus* and *Plantago lanceolata* were exposed to six exposure levels (control, 0.1, 0.3, 1, 3 and 9 ppmV). After eight days some yellow to brown spots appeared on the first trifoliate leaf of *Phaseolus vulgaris,* at the highest concentration. The concentration in the highest treatment was then increased to 15 ppmV. After 10 days the first trifoliate leaf of *Phaseolus vulgaris* became necrotic and leaves of *Plantago lanceolata* became chlorotic. No qualitative effects were observed on *Trifolium repens* or *Holcus lanatus*. The fumigation was continued for 20 days but no further effects were observed.

Based on experience from earlier studies with gaseous air pollutants (cf. Dueck *et al.*, 2003), we estimated that these concentrations were in the range of effective concentrations. These qualitative effects were assumed to result in measurable effects during a chronic exposure. Based on this range-finding test, a concentration of 27 ppmV was selected, almost the hightest concentration that could be generated, as the highest concentration to be used in the test, for which adverse effects were anticipated.

Following the range-finding test, a long-term fumigation experiment of 12 weeks was performed using six target exposure levels: control, 1, 3, 9, 15 and 27 ppmV mesitylene.

3.2 Mesitylene concentrations during plant exposure

Figure 1 shows the weekly mean mesitylene concentrations in the fumigation chambers over the exposure period. The mean measured concentrations of mesitylene in the exposure chambers and the exposure duration for each species are given in Table 2. Individual concentration measurements are given in Appendix I. The slight differences in mean concentrations for the individual species are due to differences in the length of exposure for each species. In the period between 20 and 35 days after start of the exposure some of the planned measurements were not performed due to technical problems with the GC-MS.

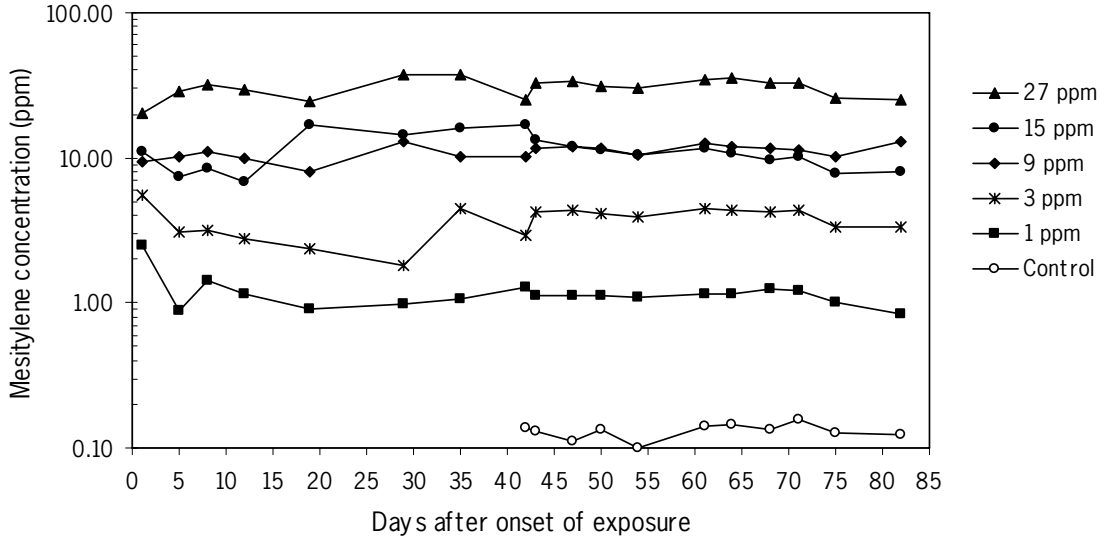

Figure 1. Measured mesitylene concentrations (ppmV) in each of the fumigation chambers during the total exposure period of 82 days. Mesitylene concentrations are shown on log scale, which is commonly used in fumigation studies.

A very low background concentration of mesitylene was initially detected in the control treatment where a mean concentration of less than 0.1 ppmV was measured up to day 40 (Fig. 1). Despite the fact that air from the climate room was directly vented outdoors, some degree of contamination in the climate room must have occurred after day 40, resulting in concentrations just above 0.1 ppmV mesitylene in the control treatment as indicated in Figure 1.

Table 2. Duration and measured mesitylene concentrations (ppmV, mean ± SE) to which plants species were exposed during the experiment.

Species	Exposure	Mesitylene concentration (ppmV)					
	(days)	control	1	3	9	15	27
Phaseolus vulgaris	41	0.14±0.004	1.27±0.14	3.25±0.31	10.22±0.37	12.23±1.14	28.87±1.54
Brassica oleracea	37	0.13±0.005	1.11±0.04	4.02±0.11	11.64±0.24	9.96±0.39	30.97±0.95
Solanum nigrum	60	0.12±0.004	1.22±0.09	3.55±0.22	10.63±0.28	12.05±0.76	29.92±1.05
Picea abies	85	0.13±0.003	1.18±0.06	3.70±0.16	11.03±0.23	11.26±0.55	30.33±0.81
Trifolium repens	60	0.12±0.004	1.22±0.09	3.55±0.22	10.63±0.28	12.05±0.76	29.92±1.05
Holcus lanatus	48	0.13±0.004	1.24±0.11	3.45±0.26	10.55±0.33	12.30±0.90	29.79±1.27
Plantago lanceolata	60	0.12±0.004	1.22±0.09	3.55±0.22	10.63±0.28	12.05±0.76	29.92±1.05

3.3 Effects of mesitylene on individual plant species

Because no dose-response relationships were found for any of the endpoints, the results presented in this report are based on the standard variation analysis, indicating the presence or absence of a (negative) trend in the treatment means. The main results per species are presented here along with a general description and conclusions. The full data set are shown in Appendix II.

Solanum nigrum (black nightshade)

Figure 2 shows the results of exposure to mesitylene on roots and shoots of *Solanum* (see also Appendix II for all values). The changes in root biomass indicate no effect of mesitylene, and although the above-ground biomass shows a tendency to increase at concentrations above 3 ppmV, no significant trend resulting from exposure to mesitylene was observed. Part of the shoot biomass is comprised of berries containing seeds, and here too, no significant (negative) trend in relationship to increasing concentrations of mesitylene was observed (Figure 3).

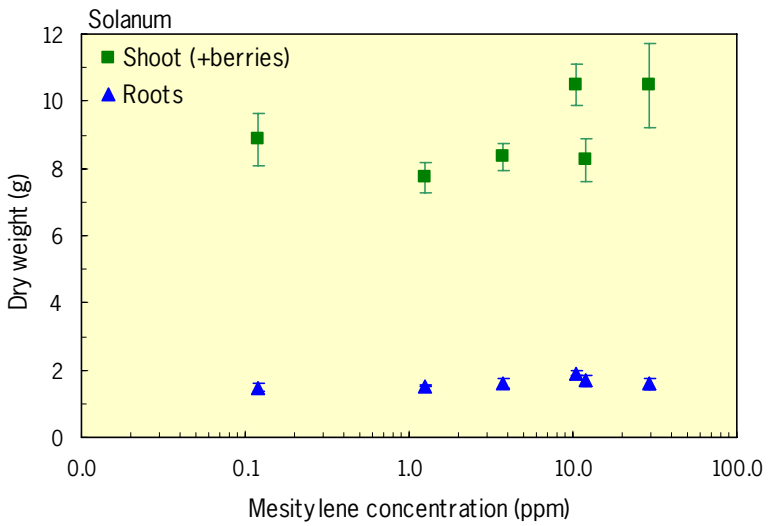

Figure 2. *Mean (± SE) shoot and root biomass (g) of Solanum nigrum after 60 days exposure to mesitylene.*

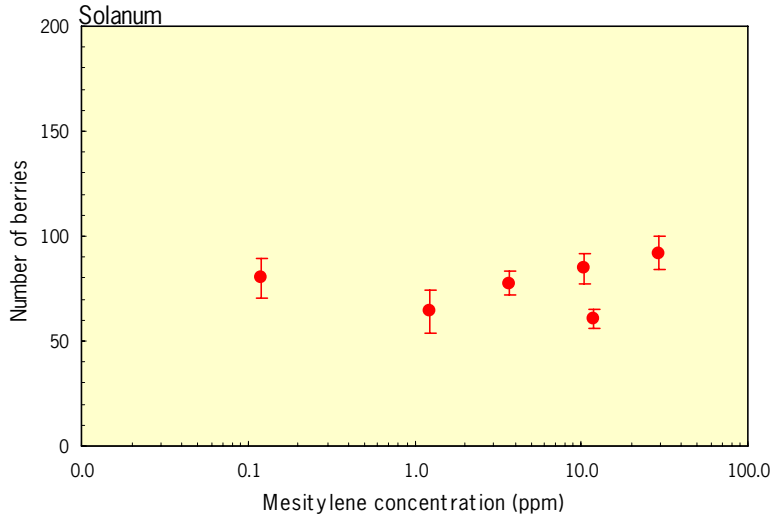

Figure 3. *Mean (± SE) number of berries of Solanum nigrum after 60 days exposure to mesitylene.*

Summary: Despite an apparent gradual trend to increasing growth (shoots) and berry production, no significant trends were noted for the measured endpoints.

Plantago lanceolata (narrowleaf plantain)

The plant biomass of *Plantago* shows a large degree of variation between individual plants. No significant effect of mesitylene on its dry weight production was observed (Figure 4).
The reproductive organs of *Plantago* , both the number (Figure 5) and length (Figure 6) of the ears appear to decrease at mesitylene concentrations in excess of 10 ppmV, although it was not statistically significant.

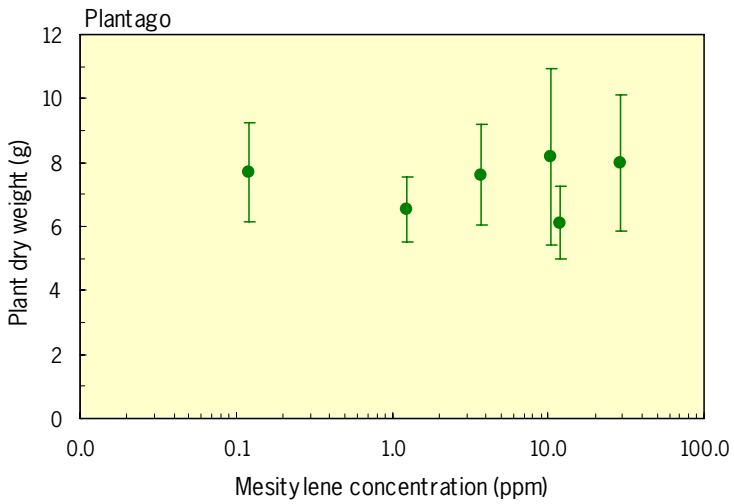

Figure 4. Mean (±SE) total biomass (g) of Plantago lanceolata after 60 days exposure to mesitylene.

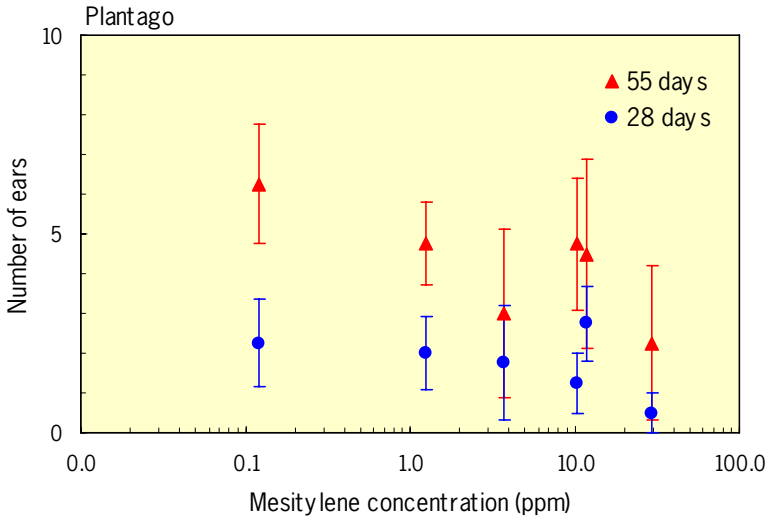

Figure 5. Mean (± SE) number of ears of Plantago lanceolata after 28, 41 and 55 days exposure to mesitylene.

Summary: Although the length and number of ears of Plantago appeared to be reduced at the higher concentrations after 28 and 55 days, no statistically significant trends were observed, indicating that mesitylene did not affect these endpoints.

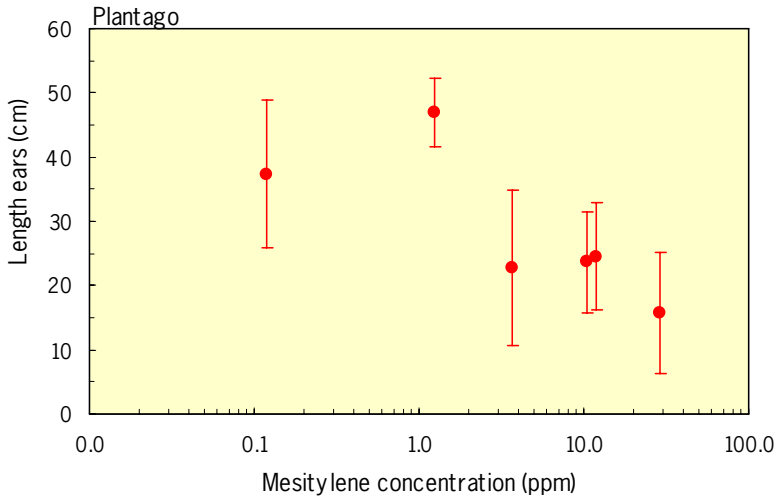

Figure 6. Mean (±SE) length of ears of Plantago lanceolata after 55 days exposure to mesitylene.

Picea abies (Norway spruce)

Picea abies showed not significant effects of increasing concentrations of mesitylene on its total biomass after almost three months (Figure 7).

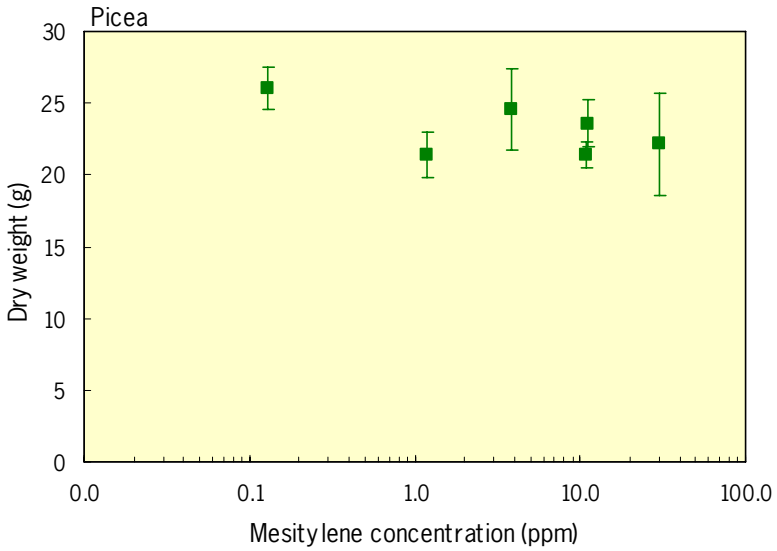

Figure 7. Mean (± SE) shoot biomass (g) of Picea abies after 85 days exposure to mesitylene.

Summary: No trend was observed for the whole plant biomass of Picea.

Brassica campestris (cabbage)

Shoot and root biomass of *Brassica* was not affected by mesitylene (Figure 8). The exposure to mesitylene did not result in a significant negative effect on the leaf area (Figure 9), even though the leaf area was much lower in three

of the four highest concentrations when compared to the control. The lack of statistical significance is possibly due to the large degree of variation among individual plants.

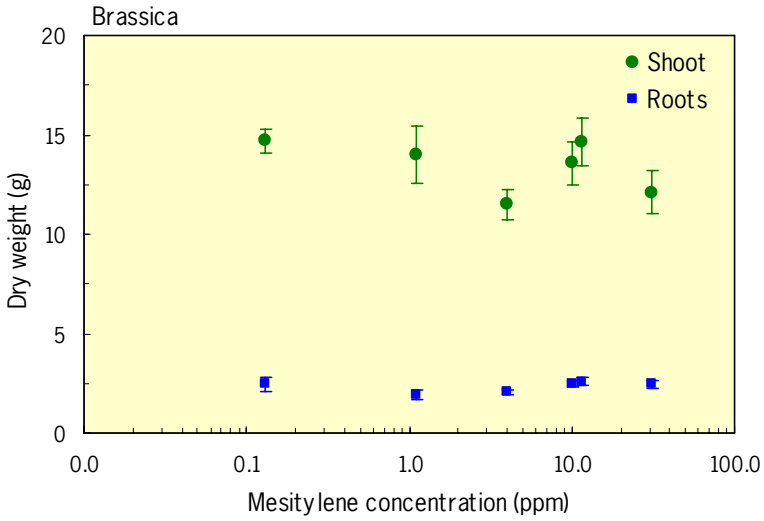

Figure 8. *Mean (± SE) shoot and root biomass (g) of Brassica campestris after 37 days exposure to mesitylene.*

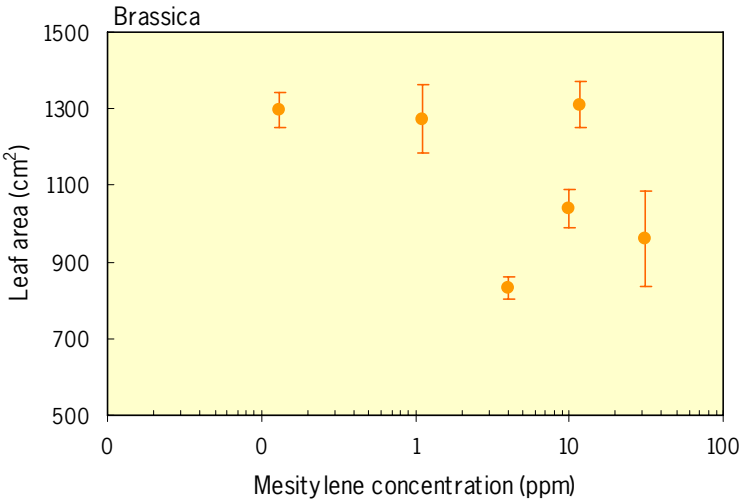

Figure 9. *Mean (±SE) leaf area of Brassica campestris after 37 days exposure to mesitylene.*

Summary: No significant negative trend was observed in Brassica for plant biomass and leaf area.

Phaseolus vulgaris **(bean)**

The qualitative, visual effects observed after 10 days in the range-finding test, were not observed in the definitive study. As was the case for *Brassica*, mesitylene does not seem to affect the biomass production of *Phaseolus*. No increase or decrease in the shoot, root or the production of pods (reproductive organs) was observed following exposure to mesitylene (Figure 10). Mesitylene did not have a significant effect on the leaf area (Figure 11) either, possibly due to the large variation between treatments.

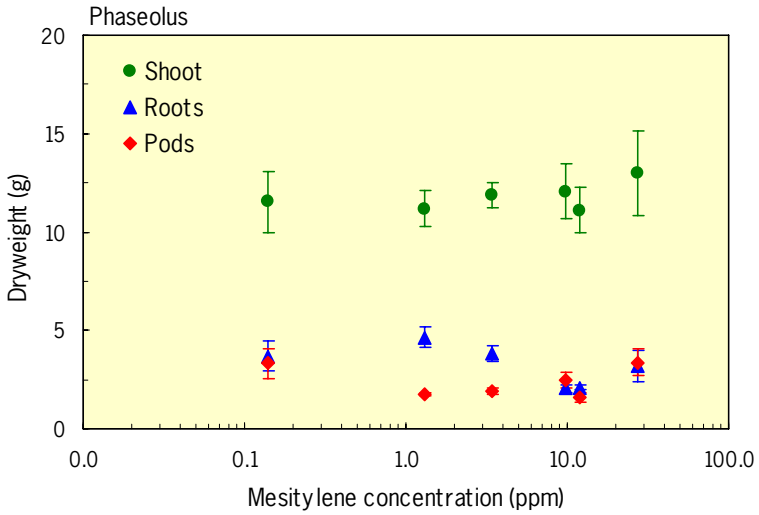

Figure 10. *Mean (± SE) shoot and root biomass (g) of Phaseolus vulgaris after 41 days exposure to mesitylene.*

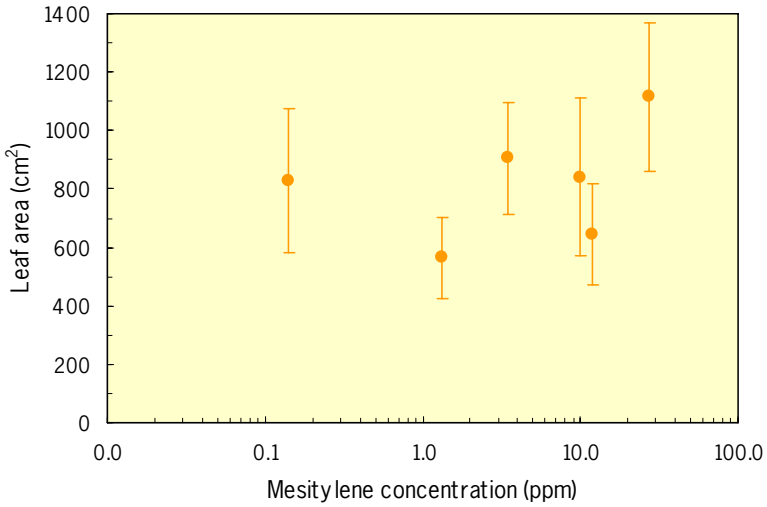

Figure 11. *Mean (± SE) leaf area of Phaseolus vulgaris after 41 days exposure to mesitylene.*

Summary: No significant trends were observed for any of the endpoints.

Holcus lanatus (common velvet grass)

The only grass among the group of tested species, Holcus lanatus, does not appear to be affected by mesitylene in any of its organs (Figure 12 and 13).

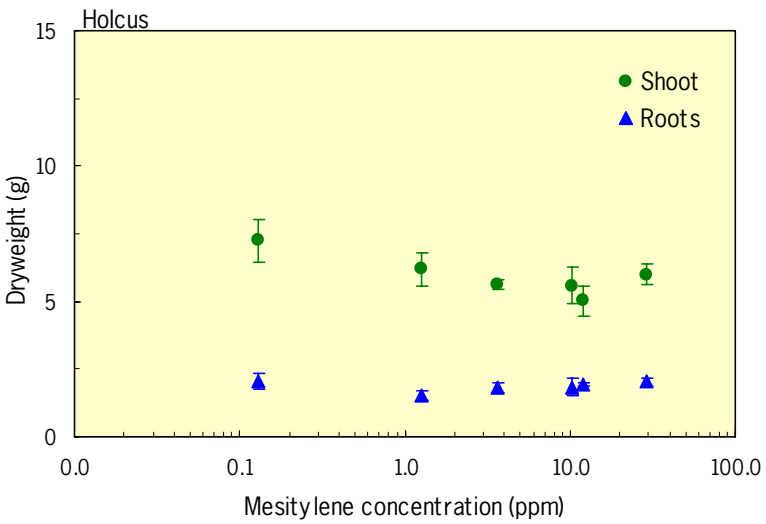

Figure 12. Mean (±SE) shoot and root biomass (g) of Holcus lanatus after 48 days exposure to mesitylene.

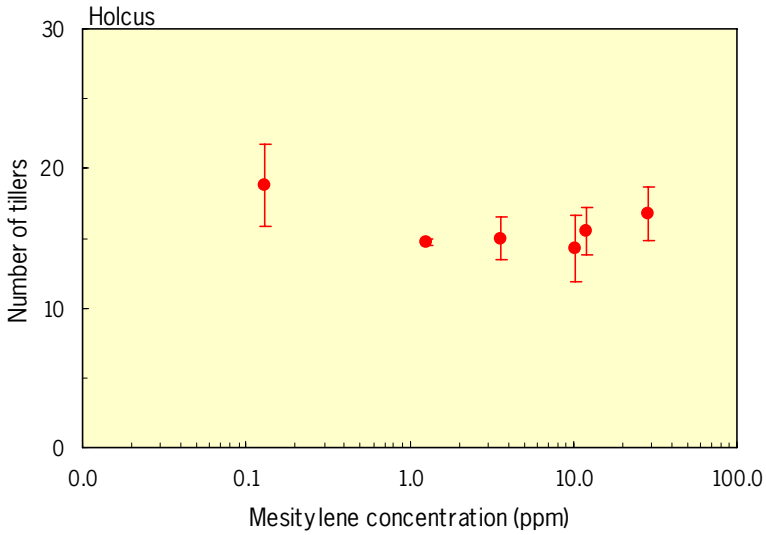

Figure 13. Mean (±SE) number of tillers of Holcus lanatus after 48 days exposure to mesitylene.

Summary: No significant trends were observed for any of the endpoints.

Trifolium repens (white clover)

No effect of mesitylene was observed on the growth of the shoots or roots of *Trifolium repens* (Figure 14). Trifolium repens flowers on newly formed stolons, which then soon produce seed or die off. Again, no significant effect of mesitylene on the flower production could be observed (Figure 15).

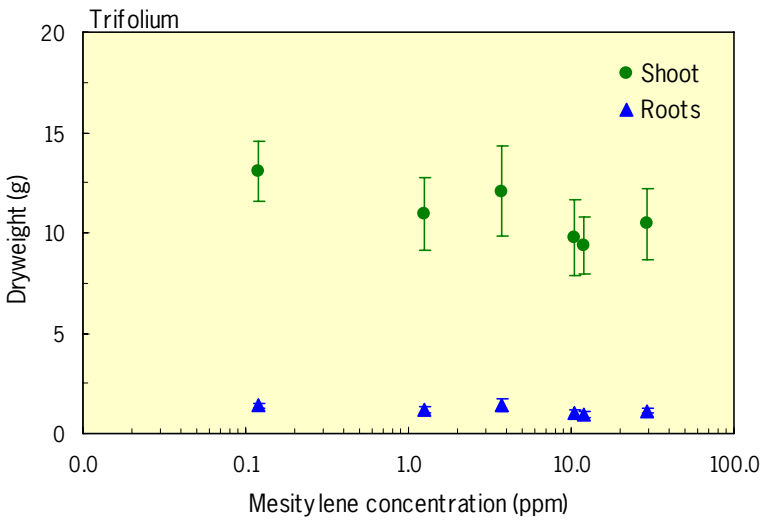

Figure 14. Mean (± SE) shoot and root biomass (g) of Trifolium repens after 60 days exposure to mesitylene.

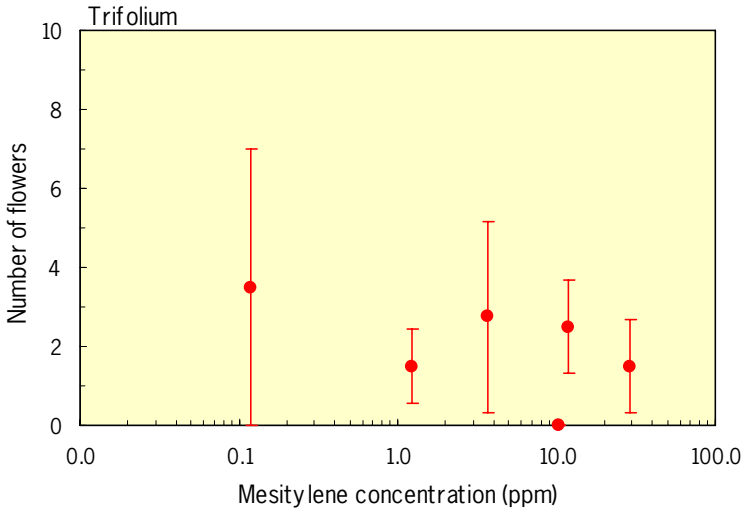

Figure 15. Mean (± SE) number of flowers of Trifolium repens after 60 days exposure to mesitylene.

Summary: No significant trends were observed for any of the endpoints.

4 Discussion

In summary, the following observations can be made:
Solanum: Despite an apparent gradual trend (visual) to increasing shoot growth and berry production, no trends were noted for the measured endpoints.
Plantago: Although the length and number of ears of Plantago appeared to be reduced at the higher concentrations, no trends were observed, indicating that mesitylene did not affect these endpoints.
Picea: No trend was observed for the whole plant biomass of Picea.
Brassica: No trend was observed in Brassica for any of the endpoints.
Phaseolus: No trends were observed for any of the endpoints.
Holcus: No trends were observed for any of the endpoints.
Trifolium: No trends were observed for any of the endpoints.

When addressing the dose-response functions for the various parameters of each of the tested plant species, the best fit from a non-linear regression approach is used, applying it in the calculation of regresson equations. The calculated NOEC would then indicate the mesitylene concentration at which the plant response differs significantly at $P<0.05$ from the calculated asymptot, or the control. However, due to the fact that even at the highest concentration of mesitylene used in this study no significant effects on the endpoints were found, NOECs could not be calculated. Even up to concentrations of almost 30 ppmV, the highest concentration of mesitylene attainable in this experimental facility, no significant trends could be observed.

This is not in line with the qualitative observations in the range-finding tests. Although some of the leaves became chlorotic after two to three weeks, the degree of chlorose remained constant. We considered that during a chronic exposure at even higher concentrations, treatment effects would be observed. The only explanation we can offer is that the visual observations during the range-finding test (chlorose) at the single highest treatment concentration for the two plant species was not related to the test substance mesitylene.

5 Conclusions

1. Based on the experimental data, no dose-response relationships could be calculated and thus no NOECs could be derived.

2. No significant (negative) trends resulting from exposure to increasing concentrations of mesitylene up to 27 ppmV could be observed for any of the endpoints.

3. Up to concentrations of 27 ppmV in ambient air, mesitylene is not toxic for the plant species investigated in this study.

References

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Appendix I.
Atmospheric mesitylene concentration

Tabel I-1. Results of the atmospheric mesitylene measurements (ppmV) in each of the fumigation chambers during the entire exposure period. Concentrations were measured twice weekly in duplicate (sample 1 and 2).

Date (2006)	Treatment (target concentration, ppmV)											
	Control		1		3		9		15		27	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
23-mrt			2.81	2.15	5.84	5.26	9.43	9.52	11.58	10.66	20.90	20.00
27-mrt			0.90	0.85	3.04	3.06	10.01	10.18	6.98	7.69	28.30	29.40
30-mrt			1.45	1.38	3.06	3.19	11.41	10.77	8.71	8.10	31.96	31.26
3-apr			1.15	1.13	2.69	2.79	9.81	10.12	6.69	6.80	28.61	30.81
10-apr			0.97	0.84	2.47	2.26	8.17	7.94	17.51	16.34	26.02	22.94
20-apr			1.05	0.93	1.90	1.74	12.54	12.98	14.33	14.20	35.85	38.81
26-apr			1.06	1.05	4.70	4.12	10.49	9.89	16.03	16.02		37.33
3-mei	0.13	0.15	1.32	1.25	2.44	3.38	8.94	11.38	15.50	18.58	28.04	22.77
4-mei	0.12	0.14	1.17	1.09	4.28	4.15	11.66	11.63	14.52	11.74	33.94	32.17
8-mei		0.11	1.10	1.13	4.34	4.27	11.94	12.13	12.65	11.35	33.75	33.08
11-mei	0.15	0.12	1.29	0.97	4.24	4.08	11.71	11.41	11.76	10.60	30.86	31.38
15-mei	0.10	0.10	1.16	1.05	3.84	3.97	10.49	10.65	11.31	9.66	29.28	30.81
22-mei	0.15	0.14	1.19	1.11	4.54	4.47	12.52	12.70	12.56	10.68	34.07	35.62
25-mei	0.15	0.14	1.04	1.29	4.36	4.32	12.25	11.81	11.72	9.73	35.57	35.16
29-mei		0.14	1.26	1.26	4.21	4.34	11.56	11.71	10.25	9.22	33.08	32.87
1-jun	0.15	0.17	1.19	1.25	4.40	4.31	11.43	11.50	11.25	9.27	33.00	32.31
5-jun	0.13	0.13	0.99	1.03	3.23	3.40	10.02	10.49	8.31	7.14	25.60	26.07
12-jun	0.12	0.13	0.77	0.90	3.20	3.42	12.09	13.88	8.14	7.83	23.86	25.9

Appendix II.
Plant measurements

Tabel II-1. Measurements of individual endpoints for each of the species subjected to a range of mesitylene concentrations for almost 12 weeks. The presence of a negative trend is indicated for each endpoint.

Treatment (ppmV)	Plant no	Solanum Shoot (g)	Roots (g)	Berries (no)	Plantago Plant (g)	Ears (no) 28 days	Ears (no) 41 days	Ears (no) 55 days	Ears (cm)	Picea Shoot (g)	Brassica Shoot (g)	Roots (g)	Leaf area (cm2)
Control	1	7.18	1.260	54	6.31	5	8	10	45.1	29.03	13.6	2.46	1180.0
	2	10.98	1.520	98	9.36	0	3	5	35.6	27.12	15.5	2.93	1402.8
	3	8.57	1.860	90	4.14	3	3	3	6.8	21.98	13.8	1.43	1290.0
	4	8.77	1.310	78	10.98	1	5	7	61.8	26.03	15.9	2.95	1312.7
1	1	8.66	1.560	72	3.99	3	4	7	42.0	19.26	11.5	1.50	1069.9
	2	8.33	1.430	60	6.27	0	4	3	62.3	22.34	12.8	1.71	1445.5
	3	7.26	1.540	87	8.92	1	4	3	44.8	18.41	18.2	2.61	1394.0
	4	6.67	1.580	38	6.96	4	5	6	38.7	27.55	13.6	1.96	1184.4
3	1	7.23	1.230	72	5.91	6	6	9	37.6	28.46	10.5	2.38	763.8
	2	8.96	1.960	93	11.32	1	3	3	48.7	30.48	12.1	1.67	866.6
	3	8.36	1.590	67	8.99	0	0	0	0.0	22.21	13.4	2.19	893.1
	4	8.84	1.670	78	4.26	0	0	0	4.5	17.59	10.1	1.93	805.3
9	1	11.94	1.920	95	5.13	3	5	7	31.1	22.66	12.4	2.62	1323.0
	2	10.52	1.900	88	6.64	0	5	5	34.3	19.63	13.8	2.04	1307.6
	3	8.93	1.740	91	4.63	2	3	7	29.1	23.11	14.5	2.57	1159.2
	4	10.51	2.040	64	16.36	0	0	0	0.0	20.78	17.9	3.03	1453.4
15	1	9.91	1.810	72	6.10	4	7	10	28.4	24.51	12.5	2.66	1188.6
	2	8.55	2.040	57	2.90	0	0	0	0.0	19.99	13.8	2.11	968.7
	3	7.70	1.610	64	8.07	4	5	7	37.5	22.92	16.6	2.75	1018.7
	4	6.83	1.270	50	7.41	3	2	1	32.0	27.68	11.4	2.36	982.7
27	1	11.71	1.490	107	10.76	2	5	8	41.9	12.28	9.9	2.43	690.9
	2	6.96	1.330	71	4.90	0	0	1	16.0	22.44	12.5	2.41	1251.4
	3	10.49	1.450	91	12.50	0	0	0	0.0	28.53	14.9	2.91	1074.5
	4	12.71	2.110	99	3.85	0	0	0	5.0	25.90	11.1	2.03	827.7
Trend		no	no	no	no	no	no	no	no	no	no	no	no

Treatment (ppmV)	Plant no	Phaseolus				Holcus			Trifolium		
		Shoot (g)	Roots (g)	Pods (g)	Leaf area (cm2)	Shoot (g)	Roots (g)	Tillers (no)	Shoot (g)	Roots (g)	Stolons (no)
Control	1	8.28	1.89	3.190	1012.93	8.36	2.83	21	16.68	1.64	0
	2	9.26	3.56	5.240	1441.04	7.24	1.98	18	14.208	1.071	14
	3	5.66	3.97	1.670	338.05	5.01	1.44	11	10.55	1.58	0
	4	9.65	5.40	3.150	521.85	8.34	1.98	25	10.82	1.25	0
1	1	6.86	4.20	1.570	214.91	7.34	1.59	14	8.24	0.84	4
	2	11.06	3.90	1.730	479.80	5.62	1.97	15	7.49	0.93	2
	3	10.00	6.19	1.810	817.33	6.50	1.45	15	14.16	1.39	0
	4	9.79	4.29	1.950	742.71	5.30	1.09	15	13.92	1.60	0
3	1	11.06	4.66	1.970	761.05	6.12	1.70	14	10.53	1.10	0
	2	8.66	4.06	1.450	502.74	5.57	2.20	19	14.45	1.78	10
	3	10.44	3.72	1.960	951.62	5.39	1.90	12	6.52	0.74	1
	4	9.73	2.85	2.290	1410.19	5.40	1.47	15	16.76	2.08	0
9	1	7.84	1.80	2.260	847.03	7.16	2.73	21	7.07	0.67	0
	2	10.55	2.06	2.200	484.80	4.83	1.40	10	12.52	1.09	0
	3	12.06	2.54	3.590	1603.21	4.19	1.47	14	13.52	1.52	0
	4	7.97	1.78	1.720	430.59	6.15	1.74	12	6.07	0.85	0
15	1	10.90	2.34	2.180	809.64	5.93	2.02	20	13.02	1.36	1
	2	8.74	1.98	1.090	415.95	3.76	1.79	13	10.02	0.87	5
	3	7.12	2.37	1.470	303.06	4.34	1.92	13	6.26	0.82	0
	4	11.42	1.75	1.540	1045.42	6.01	2.07	16	8.23	0.63	4
27	1	9.36	1.94	4.430	1117.08	6.68	1.84	12	13.64	1.42	0
	2	10.06	1.81	2.970	1121.47	6.74	2.19	21	13.36	1.22	5
	3	5.74	4.24	1.560	488.52	5.34	1.78	16	6.85	0.83	0
	4	13.19	4.78	4.580	1732.25	5.26	2.31	18	8.02	1.06	1
Trend		no	no	no	no	no	no	no	no	no	no