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New strategies for the determination of phenylurea pesticides by gas chromatography with hot splitless inlet systems

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Abstract

Direct gas chromatographic methods to analyse phenylurea pesticides are discouraged by the thermal instability of these compounds, that in conventional hot splitless inlet systems leads to extensive and irreproducible formation of isocyanates and amines. However a careful control of the operating conditions, like the inlet temperature, the pressure and the presence of suitable chemical additives (as acetic acid, low-molecular-mass amines, organic anhydrides) can either: (i) minimise the thermal decomposition enabling the direct GC–MS analysis of phenylureas, or (ii) lead to reproducible conversion to isocyanates. Experimental design was employed to study the effect of the experimental variables on the thermal transformation of phenylurea pesticides in splitless inlet system. Two strategies were alternatively optimised: (i) the minimisation of degradation reactions to increase the signal of phenylureas; (ii) the maximisation of the degradation to isocyanates that are in turn determined. The maximal yields in isocyanate were obtained with high inlet temperatures, low carrier flows in the injection phase and the presence of acetic anhydride. By contrast, the use of relatively low inlet temperatures, high carrier flows during the injection and the presence of an amine maximise the response of the parent compounds.

Keywords: Injection methods; Experimental design; Phenylureas; Pesticides

1. Introduction

Since the discovery of their herbicidal properties, phenylureas have been extensively used in agriculture as selective herbicides, mainly for preemergence weed control. Contamination of crops, soils, surface and groundwaters by residues of these compounds is of concern and several analytical methodologies were developed for their identification and quantification. High-performance liquid chromatographic analyses of phenylureas are currently utilised [1–11] with different detection techniques like UV [1–6], diode array [10], UV coupled with post column photolysis and electrochemical methods [7,8]. These methods often lack specificity and selectivity [4,5,8,18] and require a clean-up [1] of the extract or on-line enrichment process [4,10,11] to reach limits of detection (LODs) of around 10 ppt; also a micellar capillary electrophoresis method requires a preconcentration step [12]. Liquid chromatographic methods with mass spectrometric detection are also reported, with electrospray [13,14], atmospheric

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pressure chemical ionisation [15] and particle beam interfaces [16]. Gas chromatography–mass spectrometry methods are also used, because of selectivity, specificity and identification capability. It must be underlined that the thermal instability of phenylureas hampers their direct GC analysis when using hot splitless inlets. Degradation products have been reported [17–21], but the thermal reactions in the hot injector do not proceed quantitatively and are strongly influenced by the quality of liners and columns deactivation [22]. Moreover, the nature of the thermal degradation products obtained is a function of the solvents and of the impurities contained in the sample injected. From phenylureas injected in hot splitless injectors in the presence of alcohols the formation of carbamates has been reported [20], and on the other hand the feasibility of direct GC analysis has been demonstrated only for phenylureas containing a methoxy group on the nitrogen atom (e.g. *linuron*, *metobromuron, chlorbromuron*) and when using a high-quality capillary column in connection with cold on-column injection [18–22].

To overcome the thermal degradation of the phenylurea derivatives in hot injectors and columns, derivatisation methods have been developed to convert phenylureas into thermally stable compounds. The derivatisation schemes involve: (i) the hydrolysis of the phenylureas to their corresponding anilines and the subsequent derivatisation of the $-NH_2$ moiety [23], and (ii) the direct alkylation [24] or acylation [25] of the urea hydrogen. Both the approaches suffer from the drawbacks common to all derivatisation procedures; e.g. they are time-consuming and show low reproducibility.

The aim of the present work is to develop and to optimise analytical GC–MS methodologies for identification and quantitation of typical phenylurea pesticides (fenuron, monuron, isoproturon and linuron), employing conventional hot splitless injectors with programmable pressure control. Two approaches were investigated, searching for the analytical conditions that provide:

(i) the minimisation of the thermal decomposition (low inlet temperature, high carrier flow rates in the injection step, presence of an organic amine)

(ii) maximisation of the decomposition yields to isocyanates in the GC injector (high inlet temperature, presence of acetic anhydride).

The optimisation of the analytical conditions (e.g. solvent, concentration of additives, injection temperature and pressure) was performed with the aid of experimental design treatment.

2. Experimental

2.1. Materials and reagents

Fenuron (98%), *monuron* (99%), *isoproturon* (99%), *linuron* (99%) were obtained from Dr Ehresthofer (Frankfurt, Germany) and used as received. Stock solutions (500–1000 mg l^{-1}) were prepared in dichloromethane. All the solvents employed were at least of organic trace analysis grade (Suprasolv, Merck) and stored over 4 Å molecular sieve. The other reagents used were at least of analytical grade. Diethylamine was stored over 4 Å molecular sieve.

2.2. Analytical apparatus

The gas chromatographic analyses were performed on a Hewlett-Packard (HP) 5890 series II gas chromatograph, coupled with a Hewlett-Packard 5972 quadrupolar mass analyser. The gas chromatograph was equipped with a standard split/splitless inlet, an electronic pressure control and a J&W DB5 MS fused-silica capillary column (30 m×0.25 mm I.D. and 0.25 μm film thickness). The inlet is fitted with a standard HP double tapered splitless liner. Analyses were carried out with constant helium carrier flow (1.0 ml min^{-1}), except when otherwise stated.

Pressure (flow) pulse was applied during the transfer step of sample in the column (splitless time), except when long inlet residence times were used. In the last case, after an initial step with minimum flow (0.4 ml min^{-1}, corresponding to a 0.1 p.s.i. inlet pressure at 40°C) (1 p.s.i. 6894.76 N/m^2), a pressure (flow) pulse at the chosen level was applied with a duration of 1 min, then the vent valve was opened and the carrier flow set to 1.0 ml min^{-1}. During gas chromatographic analysis the septum purge flow was closed in order to prevent any possible escape of analytes through it. The oven temperature program was set at 40°C for 5 min, then brought to 280°C at

15°C/min. The mass analyser was operated in scan mode (29–350 uma). Peak areas of the analytes were obtained integrating the ion current associated to the base peak in the mass spectrum (72 for fenuron, monuron and isoproturon; 61 for linuron; 119, 153, 146 and 187 for phenylisocyanate, 4-chlorophenylisocyanate, 4-isopropylisocyanate and 2,4-diclorophenylisocyanate, respectively). The performance of the system was checked daily. After accomplishing the tune procedure of the mass analyser, the reproducibility of the abundances of the calibrant fragment ions was carefully controlled. The performances of the column and the liner were checked by injecting both the compounds prescribed by the Grob test and the mixture of the four phenylureas [splitless injection (1 min), flow pulse of 5 ml/min, inlet at 280°C]. When variations of peak areas of phenylureas and their degradation compounds exceeded 10% of the initial value recorded (new inlet liner and column), the inlet liner was replaced and 1 meter of column was cut at both ends. Differential scanning calorimetry (DSC) measurements were carried out with a Mettler Toledo model DSC12E (Schwerzenbach, Switzerland) apparatus in closed aluminium cups.

2.3. Chemometric treatment

The experiments were performed on the basis of 2-level factorial designs which allow the calculation of the effect of the experimental factors and of their interactions. The statistical treatment of the results of these designs has been extensively described elsewhere and we shall not enter into further details here [26].

An F test has been performed in order to check a possible curvature [27] of the responses. This test compares the difference between the experimental response in the centre of the domain and the predicted value with a model without quadratic terms with the experimental error:

$$F_{1,\nu,\alpha} = \frac{(Y_F - Y_0)^2}{S_{pe}^2 \cdot \left(\frac{1}{n_F} + \frac{1}{n_0}\right)} \quad (3)$$

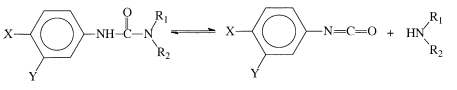
where Y_F and Y_0 are respectively the calculated and the experimental central response; S_{pe} is the purely experimental error, n_F is the number of experiments of the factorial design and n_0 is the number of the repetitions of the central experiment, ν are the degrees of freedom of the experimental error and α is the chosen level of significance of the test. On the basis of this test it result necessary to augment the design to a Hoke design [28] (central composite design with the star design experiments at the same levels of the factorial one) that permits to evaluate quadratic effect of the experimental factors.

The best OLS (ordinary least square) regression model was searched for by a forward search stepwise variables selection algorithm (STATISTICA package), with an F to enter value of 4.0. In this case the optimisation of the analysis is a multicriteria problem, since four responses are contemporary involved. The best experimental conditions were searched for by the grid search algorithm [29], i.e. the responses predicted by the regression models were calculated on a grid spanning the whole domain. The experimental settings providing the largest value of the least response (among the four ones) were selected as the optimal ones.

3. Result and discussion

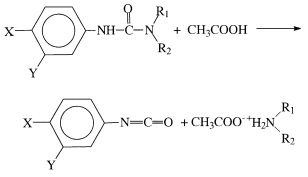
3.1. Thermal reactions of phenylureas in hot splitless GC inlet

It is well known that phenylureas are thermally unstable and undergo a variety of decomposition reactions [15,18,20], depending on the conditions and on the presence of acids, bases and active hydrogen compounds. The main equilibrium involved is the decomposition of phenylurea to give an aromatic isocyanate and an aliphatic amine (reaction 1).



(1)

The forward of reaction 1 is catalysed by acids, which can also combine with the aliphatic amine shifting the equilibrium toward the formation of the isocyanate derivatives (reaction 2).



(2)

On the other hand, the reverse reaction is catalysed by bases since likely the presence of basic compounds exert inhibitory effects on the kinetic of the forward reaction. The presence of water can also be of concern, due to rapid hydrolysis of isocyanates to aromatic amines and CO_2. Since acidic sites in the inlet liner or in the column can irreproducibily affect the extent of decomposition to isocyanates, liner and column deactivation must be carefully controlled [22]. The reaction 1 is an equilibrium, and a partial decomposition is observed even if the GC system is perfectly deactivated. A typical GC chromatogram performed in not optimised conditions shows the presence of both phenylurea and isocyanate peaks (Fig. 1). The sharpness of GC peaks associated with the decomposition products suggests that the thermal decay of phenylureas is predominantly localised in the inlet. As the inlet temperature rises, conversion to isocyanate increases.

Four phenylureas were selected as model molecule, namely fenuron, monuron, isoproturon and linuron. According to previous studies [22], monuron and isoproturon are the least thermally stable compounds, whereas linuron shows low conversion to isocyanate even at 310°C.

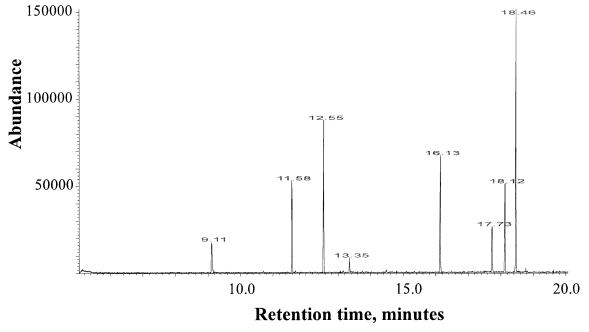


Fig. 1. Chromatogram of a phenylurea sample (10 mg l^{-1} in CH_2Cl_2) under not optimised injection conditions (Inlet temperature 280°C, carrier flow during injection 5.2 ml/min splitless 1 min). Both isocyanates and phenylureas are present. (Peak identification: 9.11 min: phenylisocyanate; 11.58 min: 4-chlorophenylisocyanate; 12.55 min: 4-isopropylphenylisocyanate; 13.35 min 3,4-dichlorophenylisocyanate; 16.13 min: fenuron; 17.73 min: monuron; 18.12 min: isoproturon; 18.46 min: linuron).

Preliminary results showed that several experimental variables (comprising GC inlet parameters, the presence of chemical additives and, presumably, even reagent impurities and matrix interference) play a role in the behaviour of phenylureas in splitless GC inlet. Also an interplay of these factors could not be excluded. The system can be optimised: (i) by avoiding the thermal decomposition of phenylureas and (ii) by maximising the conversion to isocyanates. In order to search for the experimental conditions that lead to no decomposition or to complete conversion to isocyanates, a chemometric optimisation was performed.

3.2. Chemometric optimization of the phenylurea yield

Preliminary experiments showed that the GC inlet temperature (*T*), the pressure pulse (*P*) during the injection step and the concentration of base (diethylamine, *D*) play a relevant role in the thermal transformation of the analytes. Three experimental variables must be therefore optimised to minimise the thermal degradation taking place into the GC inlet. At this purpose a two-level full factorial design [26] was employed, that requires the 8 experiments reported in Table 1. The responses are the gas chromatographic peak areas that must be maximised: the experimental data are normalised between 0 and 100.

The effects of the principal factors and of their interactions with respect to the range scaled domain (± 1) are reported in Table 2.

The models exhibit a satisfactory fitting and no evidence of non linearity of the system.

The phenylureas yield seems to be largely affected by all the parameters considered. In particular, as expected, the pressure effect is large and positive, since when the analytes are quickly transferred into the column by high initial pressure they have lower possibility to degrade. High temperature in turn favours the formation of isocyanates.

The effect of the addition of diethylamine on the yield of phenylureas is particularly important in

Table 1
Factorial design experiments (responses are normalised to 100%)

Exp.	T (°C)	P (p.s.i.)	D (%)	Fenuron	Monuron	Isoproturon	Linuron
1	220	10	0	14.00	6.20	4.70	17.50
2	280	10	0	30.20	30.00	31.90	41.70
3	220	36	0	46.90	21.10	19.50	34.40
4	280	36	0	59.50	74.10	71.10	69.60
5	220	10	1.0	28.10	10.90	9.80	27.00
6	280	10	1.0	34.70	23.50	31.10	47.40
7	220	36	1.0	100.00	100.00	100.00	100.00
8	280	36	1.0	36.60	23.10	31.30	39.70
Centre	250	23	0.5	48.30±3.54	32.9±3.46	34.4±3.85	46.0±4.03

interaction with temperature. This is the second effect, in order of importance. The interaction effect is always negative, showing that an antagonistic effect of *T* and *D* takes place.

By means of the optimisation grid search algorithm the conditions that maximise the yields were searched for by maximising the lowest of the four yields, calculated with the regression models. The resulting optimal settings were: 220°C for the initial temperature of the injector, 36 p.s.i. for the pressure of the carrier with the addition of 1% (w/w) diethylamine. These experimental conditions, corresponding to experiment 7 of the factorial design (Table 1) showed indeed that isocyanates formation is precluded and, at the same time, the phenylureas concentrations is maximised.

From the comparison of the chromatogram in Fig. 1 with that obtained in the optimised condition (Fig. 2) it is possible to note the absence of the characteristic peaks of the isocyanates and the increasing abundance of the phenylurea peaks. The only drawback of this approach is the appearance of a fifth peak, due to the reaction product between phenylisocyanate and diethylamine. However, the relative standard deviation of peak areas of fenuron calculated from three result respectively obtained under the optimised conditions, in two experiment performed at concentration levels of 1.0 and 10 mg l^{-1} respectively, is under 5%.

LODs at $S/N=3$ are 0.30, 0.35, 0.25 and 0.15 mg l^{-1} for fenuron, monuron, isoproturon and linuron, respectively. Lower LODs can be achieved operating

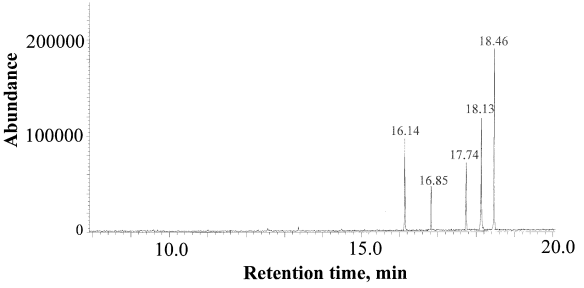


Fig. 2. Chromatogram obtained in the conditions optimised to minimise phenylurea derivatives degradation (10 mg l^{-1} each in CH_2Cl_2, Diethylamine 1%, inlet temperature 220°C, splitless 1 min, carrier flow during injection 5.2 ml min^{-1}). The peak at 16.85 min is *N,N*-diethyl-*N′*-phenylurea (see text for detail). Peak identification as in Fig. 1.

Table 2
Factor effects (in bold the relevant factors at 95% confidence level)

Compound	Offset	*T*	*P*	*TxP*	*D*	*TxD*	*PxD*	*TxPxD*
Fenuron	**43.75**	−7.00	**34.00**	**−18.40**	12.20	**−21.40**	2.90	**−16.60**
Monuron	**36.11**	3.13	**36.93**	**−15.08**	6.53	**−35.27**	7.42	**−29.67**
Isoproturon	**37.43**	7.85	**36.10**	**−16.40**	11.25	**−31.55**	9.10	**−28.60**
Linuron	**47.16**	4.88	**27.53**	**−17.43**	12.73	**−24.82**	5.12	**−22.92**

the mass analyser in selected ion monitoring mode. The linear correlation coefficients of the calibration curves are better than 0.998 in the concentration interval 1.0–25.0 mg l^{-1}.

3.3. Chemometric optimisation of the isocyanates yield

In the second approach an indirect determination of the phenylureas through the determination of the isocyanates was optimised. In this case five experimental variables possibly influence the response:

(i) the inlet temperature (T);
(ii) the carrier gas pressure (flow) during the injection step (P);
(iii) the analytes residence time in the inlet (R);
(iv) the water concentration (simulation of solvents containing trace water) (H);
(v) the acetic anhydride concentration (AA).

Since a full factorial experimental design for five variables requires $2^5=32$ experiments, a 2^{5-2} fractional factorial design, which requires only eight experiments, was initially used (Table 3). The analytical responses given as the ratio between each gas chromatographic peak area with respect to the largest peak, taken as 100, are reported for each analyte in the first 8 rows of Table 3.

An F-test, performed in the centre of the variable dominion, showed a significant difference between the estimated and the experimental responses to indicate the evidence of curvature. Therefore a second order regression model was necessary and a star design was added to the fractional experimental design. Since the experimental domain cannot be further enlarged, the star design experiments were performed at the same levels of the fractional factorial design (Table 3, Hoke design).

The independent variables were scaled in the range ± 1 before calculating the regression models. The best OLS models were searched from the data of Table 3, using a forward search step-wise variables selection algorithm (F to enter=4.00). The coefficients of the final models are reported in Table 4. All models show satisfactory descriptive capabilities with R^2 values greater than 0.85–0.90. The most important factors are usually T and R and the interaction $P \times H$. The regression models were used

Table 3
Experiments of fractional factorial design (FrFD), centre and star design (responses are normalised between 0 and 100)

Esp.	T (°C)	P (p.s.i.)	R (min)	H (%)	AA (%)	Phenyl isocyanate	Chlorophenyl isocyanate	Isopropyl phenyl isocyanate	Dichloro phenyl isocyanate
FrFD	220	10	0	1	0.10	53.4±7.3	49.8±15.4	52.0±13.6	30.7±18.9
FrFD	280	10	0	0	0	83.0±11.0	85.0±15.5	77.6±16.0	68.5±11.3
FrFD	220	35	0	0	0.10	54.5±8.6	65.9±29.0	64.8±30.6	30.5±12.5
FrFD	280	35	0	1	0	82.6±18.6	71.8±33.5	85.4±25.3	37.4±14.3
FrFD	220	10	1	1	0	27.7±12.7	22.5±10.9	35.3±18.7	19.1±8.6
FrFD	280	10	1	0	0.10	100.0±0.0	94.7±9.2	92.4±8.1	100.0±0.0
FrFD	220	35	1	0	0	34.3±14.0	31.8±8.2	33.3±6.3	34.3±13.9
FrFD	280	35	1.0	1.0	0.10	72.7±11.1	58.9±13.6	59.6±8.9	73.3±2.4
Centre	250	22.5	0.5	0.5	0.05	27.4±0.3	26.1±4.5	25.9±5.0	19.9±2.9
Star	220	22.5	0.5	0.5	0.05	27.8±10.6	31.1±8.3	28.8±7.8	22.2±7.3
Star	280	22.5	0.5	0.5	0.05	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Star	250	10	0.5	0.5	0.05	36.5±24.0	35.9±21.4	33.6±18.9	33.4±17.5
Star	250	35	0.5	0.5	0.05	39.5±9.2	46.3±12.8	43.1±10.5	31.9±7.3
Star	250	22.5	0	0.5	0.05	73.7±16.4	85.8±14.1	73.8±9.4	46.8±5.9
Star	250	22.5	1.0	0.5	0.05	69.6±4.9	69.5±11.2	66.9±12.0	66.7±0.8
Star	250	22.5	0.5	0	0.05	50.7±9.2	57.2±12.7	49.4±12.2	44.0±4.2
Star	250	22.5	0.5	1.0	0.05	21.4±7.0	20.7±4.1	18.5±3.6	10.8±3.1
Star	250	22.5	0.5	0.5	0	28.9±2.3	32.4±1.9	32.7±2.7	18.0±3.3
Star	250	22.5	0.5	0.5	0.10	46.0±6.0	46.5±5.8	42.0±6.2	36.1±4.8

Table 4
Regression models for the isocyanates yield optimisation (range scaled domain)

Compound	R^2	Regression models
Phenyl isocyanate	0.9106	$42.6+36.1\,T+24.9\,R^2+7.0\,AA-6.5\,H-15.0\,P^*H+17.1\,T^2-10.8\,H^2-9.3\,AA^2$
Chlorophenyl isocyanate	0.9095	$46.7+34.5\,T+25.9\,R^2-11.1\,H-8.1\,R+7.2\,AA-12.8\,H^2-16.9\,P^*H+13.8\,T^2-12.3\,AA^2$
Isopropylphenyl isocyanate	0.8875	$41.5+35.6\,T-21.7\,R^2-19.4\,P^*H-6.7\,H-7.3\,T^*AA-6.9\,P^*R+15.7\,T^2-14.6\,H^2$
Dichlorophenyl isocyanate	0.8379	$34.2+38.9\,T+17.4\,T^2-10.6\,H+9.3\,AA+7.9\,R-18.3\,P^*H$

for optimising the system by the grid search algorithm. The result of the optimisation procedure gives the maximum yields for phenyl isocyanate, *p*-chlorophenylisocyanate, *p*-isopropylphenylisocyanate and 2,4-dichlorophenylisocyanate for the following experimental conditions: $T=280$°C, $P=35$ p.s.i., $R=1$ min, $H=0$ and $AA=0.1\%$. Fig. 3 shows a chromatogram recorded under the optimised conditions: as expected, the signals for phenylurea are below the detection limits.

For isocyanate derivatives the linear correlation coefficients of the calibration curves are better than 0.998 in the concentration interval 1.0–20.0 mg l^{-1}.

The limits of detection respectively for phenylisocyanate, 4-chlorophenylisocyanate, 4-isopropylphenylisocyanate and 2,4-dichlorophenylisocyanate, at $S/N=3$ are: 0.70, 0.30, 0.25 and 0.45 mg l^{-1} expressed as concentrations of phenylurea injected. Lower LODs can be achieved operating the mass analyser in selected ion monitoring mode.

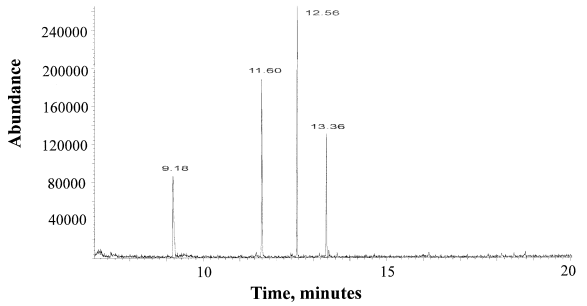


Fig. 3. Chromatogram obtained in the conditions optimised to maximise isocyanate yields (10 mg l^{-1} each in CH_2Cl_2, acetic anhydride 0.1% (w/w), inlet temperature 280°C, splitless 2 min, carrier flow during injection 0.4 ml min^{-1} for 1 min, then 5.2 ml min^{-1} for 1 min). Peak identification as in Fig. 1.

4. Conclusions

Phenylurea derivatives show, in hot splitless inlets, a complex thermal degradation behaviour, that is not only influenced by inlet operating parameters, like temperature and carrier flow during the injection step, but also by the presence of acidic or basic chemical additives. It follows that not controlled acidic or basic impurities present in the solvent can lead to unexpected variations in the gas chromatographic behaviour of phenylureas. With the aid of experimental design techniques we were able, for their determination, to find the optimal conditions to: (i) minimise thermal degradation of phenylurea derivatives, or alternatively (ii) maximise their conversion to isocyanates.

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