Transfer of Maleic Hydrazide into Mainstream Cigarette Smoke*

by

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SUMMARY

This report presents the results of a study regarding the transfer of maleic hydrazide (MH) into mainstream cigarette smoke. Cigarettes with different levels of MH were used in this study. This included cigarettes with MH preexistent in the tobacco due to the agronomical practice, and with spiked MH. Because the MH can be present in tobacco as bound and free forms, both levels of MH were measured in the tobacco section. The cigarette designs covered a range of possibilities, including Plain, Filter King Size (KS), Lights 100's, Ultra Lights, etc. The results showed that the amount of MH in smoke, on the one hand, is a function of the total particulate matter (TPM) of the cigarette, and higher TPM levels lead to more MH in smoke. On the other hand, the transferred level of MH depends on the total amount of MH (both bound and free) in the tobacco. The relative % transfer is higher for lower MH levels than for higher MH levels in tobacco. When normalized by TPM, the transfer as an average is about 0.24% per mg of TPM from the amount of MH in 1 g of tobacco, and as high as 0.46% per mg of TPM from the amount of MH in 1 g tobacco for a nonfilter low MH level cigarette. The resulting MH transfer for a nonfilter cigarette with low tobacco MH is therefore about 8.3% from the total MH in the cigarette. For filter full flavor (FF) cigarettes with high tobacco levels of MH, the transfer is about 5.8%. This relative transfer rate appears to be lower from higher MH levels in tobacco. [Beitr. Tabakforsch. Int. 20 (2003) 325–330]

ZUSAMMENFASSUNG

Dieser Bericht fasst die Ergebnisse einer Studie über den Transfer von Maleinsäurehydrazid (MH) in den Hauptstromrauch von Zigaretten zusammen. In dieser Studie wurden verschiedene Zigaretten mit unterschiedlichen MH-Konzentrationen untersucht, darunter sowohl Zigaretten, bei denen MH bereits im Tabak aufgrund agronomischer Praktiken vorhanden war, als auch Zigaretten, die mit MH dotiert wurden. Da MH im Tabak sowohl in gebundener als

auch in freier Form vorliegen kann, wurden die Konzentrationen beider MH-Fractionen im Tabak gemessen. Eine breite Palette von Zigarettenarten wurde untersucht, einschließlich Zigaretten ohne Filter, Filter King Size (KS), Lights 100's, Ultra Lights, etc. Die Ergebnisse zeigen, dass die Menge des MH im Zigarettenrauch einerseits als Funktion der im Rauch enthaltenen Gesamtpartikelmasse (TPM) dargestellt werden kann; je höher die TPM Menge, umso mehr MH liegt im Zigarettenrauch vor. Die Gesamtmenge des transferierten MH hängt andererseits von der Gesamtmenge an MH (sowohl gebunden als auch frei) im Tabak ab. Der relative prozentuale Transfer ist bei niedrigen MH-Konzentrationen höher als bei hohen MH-Konzentrationen. Bezogen auf TPM beträgt der Transfer durchschnittlich ca. 0,24% der in 1 g Tabak enthaltenen MH-Menge pro mg TPM; bei filterlosen Zigaretten mit niedrigen MH-Konzentrationen kann der Transfer auf bis zu 0,46% der in 1 g Tabak enthaltenen MH-Menge pro mg TPM ansteigen. Dies bedeutet, dass bei einer filterlosen Zigarette mit niedriger MH-Konzentration ca. 8,3% der Gesamtmenge des in der Zigarette enthaltenen MH transferiert werden. Bei "Full Flavour" (FF) Filterzigaretten mit hohen MH-Konzentrationen werden ca. 5,8% transferiert. Diese relative Transferrate scheint bei höheren MH-Konzentrationen im Tabak niedriger zu sein. [Beitr. Tabakforsch. Int. 20 (2003) 325–330]

RESUME

Ce rapport présente les résultats d'une étude sur le transfert de l'hydrazide maléique (MH) dans la fumée du courant principal des cigarettes. Dans cette étude, des cigarettes avec différentes teneurs en MH ont été utilisées. Certaines de ces cigarettes comportaient du MH préexistant dans le tabac par pratique agronomique, alors que chez d'autres le MH a été apporté. En raison du fait que le MH peut apparaître dans le tabac sous forme libre ou conjugué, les deux teneurs de MH ont été mesurées dans la colonne de tabac. L'étude inclut des cigarettes de diverses conceptions dont des cigarettes sans filtre, des cigarettes extra longues (KS) avec filtre, des cigarettes légères 100's, des cigarettes ultra-légères, etc. Les

résultats de l'étude ont révélé que la quantité de MH présente dans la fumée est, d'une part, fonction de la matière particulaire totale (MPT) de la cigarette et que plus les teneurs en MPT sont élevées plus il y a de MH dans la fumée. D'autre part, la teneur de MH transférée dépend de la quantité totale de MH (conjuguée et libre). Le pourcentage relatif de transfert est plus élevé pour des teneurs basses en MH que pour des teneurs élevées. Lorsqu'on le rapporte à la MPT, le transfert de MH s'élève en moyenne à environ 0,24% de la quantité de MH présente dans 1 g de tabac par mg de MPT, et peut atteindre 0,46% de la quantité de MH présente dans 1 g de tabac par mg de MPT pour une cigarette sans filtre à faible teneur en MH. Le transfert de MH d'une cigarette sans filtre à faible teneur en MH est donc environ 8,3% du MH total dans la cigarette. Pour les cigarettes à filtre plein arôme (FF) ayant des teneurs élevées en MH, le transfert s'élève à environ 5,8%. Le pourcentage relatif de transfert semble être faible pour des teneurs plus élevées en MH dans le tabac. [Beitr. Tabakforsch. Int. 20 (2003) 325–330]

INTRODUCTION

Maleic hydrazide (MH) is a synthetic plant growth regulator widely used in tobacco farming as a suckering control agent. MH is typically applied on the upper part of tobacco plants after topping. In time, the MH becomes distributed through the whole plant where it is present both as unmodified MH and MH metabolites. One of the typical MH metabolites is a β -D-glucoside (1). The total level of MH in the processed leaf and in the tobacco from cigarettes ranges between a few $\mu\text{g}/\text{cig}$ up to about 100 $\mu\text{g}/\text{cig}$. Pyrolysis at 600 °C of pure MH, performed in the present study, indicates that at elevated temperatures some MH is transferred unmodified into the pyrolysate. The transfer of MH from tobacco to cigarette smoke is therefore likely. Several previous studies report MH transfer into smoke. LIU and HOFFMANN were the first to report that the transfer rate of MH depends on the concentration of MH in tobacco (2). The transfer rate indicated by LIU and HOFFMANN (2) ranged from 4% to 10%. HAEBERER and CHORTYK (3) found a transfer rate of only 0.2%. A report by the U.S. Department of Agriculture (4) indicated a transfer of 2–3% of MH to mainstream smoke, while CHOPRA *et al.* (5), showed values between 2.5% and 2.8%. Additionally, Liu *et al.* (6) indicated that MH is present in smoke even if the tobacco was not treated with MH.

The variability in the reported results on MH transfer is complicated by the fact that the analysis of MH in plant material must capture both free and bound MH. To accomplish the analysis of total MH, a hydrolysis of bound MH must be performed. Typically this is done with aqueous HCl and heating (7–12). This hydrolysis requires rather strong conditions, and concentrations of HCl between 2 and 12 *N* are used in different methods. The ISO 4876 method and the official method of the Association of the Official Analytical Chemists apply an acidic digestion followed by hydrolytic reduction of the free MH to hydrazine with Zn in aqueous NaOH. Hydrazine is steam distilled into a *p*-dimethylamino-benzaldehyde solution and the resulting reaction product analyzed spectrophotometrically (13–15).

The disagreement between the reported results of MH trans-

fer indicated a need for further studies. The present work was done to clarify the reasons for differences among previously published results. It started with the pyrolysis of pure MH to establish the capability of MH to transfer unmodified into pyrolysates. Then, measurements of MH on tobacco and smoke were performed. The procedure for MH analysis in tobacco was taken from the literature (11) and an original procedure based on derivatization with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) for the analysis of MH in smoke condensate was applied. Several cigarette brands with tobacco having various levels of MH were evaluated and the results are compared to those reported in the literature.

EXPERIMENTAL

The materials used for the experimental part of this study are as follows: MH from Chem Services Inc. (West Chester, PA, USA), BSTFA from Regis Technologies Inc. (Morton Grove, IL, USA), dimethylformamide (DMF) from Burdick and Jackson (Muskegon, MI, USA), *tert*-butylhydroquinone and docosanoic acid from Aldrich (Saint Louis, MO, USA). The pyrolysis of pure MH was done using two different systems, an on-line pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) and an off-line system. The on-line system consisted of a CDS AS-2500 pyrolysis autosampler, connected to a CDS 2000 pyroprobe, and an Agilent 6890-5973 GC-MS system. The sample was pyrolyzed in the pyroprobe at 600 °C for 10 sec, and the pyrolysates were transferred to the GC-MS system for analysis. The GC was equipped with a 60 m long, 0.25 mm i.d. and 0.5 μm film thickness DB-Wax capillary column. The oven was programmed at an initial temperature of 40 °C for 2 min, a heating rate of 2 °C/min to 240 °C, and a final time of 20 min.

For the off-line system a Pyroprobe 100 was used. The pyrolysis was performed in similar conditions as those used for on-line analysis and the collection and derivatization of pyrolysate was done by a procedure reported previously (16). In this procedure, the pyrolysate is collected in a 1-m length of 0.53-mm i.d. untreated fused silica tube. Approximately 0.25 m from the exit end of the silica tube, a loop of the tube is immersed in ice water. After pyrolysis, the tube is flushed with helium for 1 min. The tube is then removed from the ice water bath and the ends inserted into two sealed GC autosampler vials. One of the vials contains a mixture of 80 μL BSTFA and 40 μL DMF. Using gas pressure, the liquid was passed through the collection tube several times to wash the pyrolysate. After washing, the solution is heated for 30 min at 76 °C and analyzed using a GC-MS system. The GC is equipped with a 20 m long, 0.18 mm i.d. and 0.25 μm film thickness DB-5 capillary column. The oven is programmed at an initial temperature of 50 °C for 2 min, a heating rate of 5 °C/min to 200 °C, and 8 °C/min to 300 °C and a final time of 12 min. The injection is done at 275 °C. MH is present in several tautomeric forms and in the presence of BSTFA, the enol form reacts as shown in Figure 1.

The mass spectrum of silylated MH or 3,6-bis[(trimethylsilyl)oxy]-pyridazine is similar to that of silylated 2,5-dihydroxypyrazine and is shown in Figure 2. The quantitation was done using the area of the MH peak in the total ion chromatogram.

Figure 1. Tautomeric forms of maleic hydrazide and the reaction with *N,O*-bis(trimethylsilyl)trifluoroacetamide

The analysis of MH in smoke is done after 5 cigarettes are smoked onto a small Cambridge pad, under (Federal Trade Commission (FTC) conditions using a Borgwaldt RM-20/CS smoking machine. The smoke pad is then extracted with 10 mL methanol on a mechanical shaker for 20 min. The solution is transferred into a test tube, heated at 65 °C under a N₂ stream, and the methanol is evaporated to dryness. The residue is dissolved in 1 mL DMF containing 400 µg/mL *tert*-butylhydroquinone + 200 µg/mL docosanoic acid, followed by the addition of 0.5 mL BSTFA. The solution is heated at 76 °C for 30 min and analyzed by GC-MS using conditions similar to those used to analyze the pyrolysate. Some cigarettes were spiked with different levels of MH. For this purpose, a 10 or 20 µL solution of MH was injected, and the spiked cigarettes were equilibrated for 10 d before smoking. The concentration of MH in methanol was prepared to yield the desired amount of MH in the cigarette. No further verification of the achieved MH levels on the cigarettes was done.

The quantitation of MH in cigarette smoke is done following a calibration curve made using standards of pure MH. The standard solutions used to make the calibration curve are prepared starting with a stock solution of 1 mg/mL MH, 400 µg/mL *tert*-butylhydroquinone (internal standard no.1)

Figure 3. Calibration curve for the determination of MH in smoke

and 200 µg/mL docosanoic acid (internal standard no. 2) in *N,N*-dimethylformamide (DMF). This solution is diluted to 200 µg/mL MH with DMF containing the same concentration of internal standards, and various volumes from this solution were further diluted to generate each point. Three replicates of each concentration were prepared to generate the calibration curve. The calibration curve for MH in smoke is shown in Figure 3.

The analysis of MH in tobacco is done following two different procedures. The first procedure is developed to analyze only the free tobacco MH. For this purpose, 250 mg of ground tobacco is extracted with 1 mL methanol in a 2 mL capped vial by heating at 76 °C for 30 min. The vial is then cooled to room temperature and the liquid material from the vial is separated by centrifugation and filtration. The liquid is transferred into a vial, heated at 65 °C under a N₂ stream, and the methanol is evaporated to dryness. The residue is analyzed like the smoke extract. For quantitation, tobacco free of MH was spiked with various levels of MH and processed as previously described. The quantitation is done using the calibration curve shown in Figure 4.

The repeatability of the procedure has been tested near the lower limit of measurement and the results are shown in Table 1. Taking as detection limit the value of 3 SD, the

Figure 2. Mass spectrum of 3,6-bis[(trimethylsilyl)oxy]-pyridazine (MH-2 TMS)

Figure 4. Calibration curve for the determination of MH in tobacco.

Table 1. Study of the repeatability of the MH determination in the tobacco of a commercial filter KS cigarette with low MH level

$\mu\text{g MH/g tobacco}$	$(x_i - x_{\text{mean}})^2$
9.85	0.013
9.24	0.239
10.59	0.728
9.07	0.446
10.14	0.168
9.51	0.049
Mean = 9.73	SD ^a = 0.57

^aSD = standard deviation.

Table 2. The level of total MH, free MH, and MH in mainstream smoke on a per-cigarette basis for various non-spiked and MH-spiked cigarette brands

Cigarette brands	Total MH ($\mu\text{g/cig}$)	Free MH ($\mu\text{g/cig}$)	MH in smoke ($\mu\text{g/cig}$)	TPM (mg/cig)	g Tobacco (in 1 cig)
<i>Non-spiked samples</i>					
Experimental KS A	0.0	2.5 ^a	0.30	21	0.649
Plain end (no filter) KS	12	5.8	1.00	18	0.960
Full Flavor KS	8.4	3.7	0.87	17	0.697
Ultra Lights 100's	9.4	5.9	0.24	5.8	0.784
Lights 100's	9.5	6.5	0.63	11	0.794
Menthol Mild KS	26	9.8	1.30	13	0.650
Menthol 100's	33	12	2.30	22	0.819
Experimental KS B	79	26	3.30	19	0.789
1 mg TPM cig 100's	18	5.3	0.07	1	0.679
<i>MH-spiked samples</i>					
Menthol 100's + 20 $\mu\text{g/cig}$ MH	58	35.8	2.30	19	0.819
Experimental KS B + 20 $\mu\text{g/cig}$ MH	103	50.7	3.90	19	0.789
Experimental KS A + 6.5 $\mu\text{g/cig}$ MH	6.5	6.0	0.29	20	0.649
Experimental KS A + 13 $\mu\text{g/cig}$ MH	13	11.5	0.74	19	0.649
Experimental KS A + 26 $\mu\text{g/cig}$ MH	26	24.0	1.30	19	0.649

^aNote: The result for total MH in the experimental KS A cigarette is 0.0 $\mu\text{g/cig}$, while the result for free MH is 2.5 $\mu\text{g/cig}$. The bound and free MH were measured by different analytical techniques, and this discrepancy is within experimental error of the two methods.

results shown in Table 1 indicate a detection limit of about 1.5 $\mu\text{g/g}$ of tobacco.

The analysis of total MH was performed by an external laboratory. The method used for this analysis is similar to that described in a literature report (11). For the analysis, 5 g of tobacco was extracted by boiling under reflux in 100 mL of 4 N HCl for 40 min. The extract is allowed to cool and is filtered. A few mL of solution are passed through a C-18 SPE column and a 3 mL sample is collected. A 10 μL aliquot is injected in a high performance liquid chromatography (HPLC) system equipped with a C-8 column. The elution is done using isocratic conditions with 0.3% w/v H_3PO_4 in water (pH $\approx$ 2) at 0.5 mL/min. The optical density is measured at 320 nm.

RESULTS AND DISCUSSION

The result of MH pyrolysis indicates that a significant amount of MH passes unchanged into the pyrolysate. The relatively high thermal stability of MH and its volatility

allowed for part of the MH to volatilize and leave the pyrolyzer before decomposition. The decomposed part of MH consisted of some ammonia, CO_2 , butanoic acid, aminobutyric acid, traces of acetonitrile, acrylonitrile, 1H-pyrrole-2,5-dione, etc. This result indicates that besides MH itself, no specific toxicological concerns are raised by the presence of MH in cigarettes. To obtain quantitative information on the transfer of MH in smoke, it was necessary to determine the levels of MH in both the tobacco and the smoke of each type of cigarette.

Cigarettes with different levels of MH were used in this study. This included cigarettes with agronomically added MH in the tobacco, and with spiked MH. Because the MH can be present as bound and free forms, both levels of MH were measured in the tobacco section. The cigarette designs covered a range of possibilities, including Plain, Filter King-Size (KS), Lights 100's, Ultra Lights, etc. cigarettes. The level of total MH, free MH, and MH in smoke were analyzed as previously described. The results are given in Table 2 and are all expressed on a per-cigarette basis. The data from Table 2 were further processed by normalizing the

Table 3. The level of MH/TPM in mainstream smoke and the MH levels in 1 g tobacco for various non-spiked and MH-spiked cigarette brands

Cigarette brands	µg MH in smoke/ mg TPM	Total µg MH/ g tobacco	Free µg MH/ g tobacco
<i>Non-spiked samples</i>			
Experimental KS A	0.014	0	3.9 ^a
Plain end (no filter) KS	0.058	12	6.0
Full Flavor KS	0.052	12	5.3
Ultra Lights 100's	0.042	12	7.6
Lights 100's	0.056	12	8.2
Menthol Mild KS	0.100	40	15.0
Menthol 100's	0.104	40	14.0
Experimental KS B	0.172	100	33.0
1 mg TPM cig 100's	0.066	26	7.9
<i>MH-spiked samples</i>			
Menthol 100's + 20 µg/cig MH	0.121	71	44.0
Experimental KS B + 20 µg/cig MH	0.200	131	64.0
Experimental KS A + 6.5 µg/cig MH	0.015	10	9.2
Experimental KS A + 13 µg/cig MH	0.039	20	18.0
Experimental KS A + 26 µg/cig MH	0.069	40	37.0

^aNote: The result for total MH in the tobacco for the experimental KS A cigarette is 0.0 µg/g while the result for free MH is 3.9 µg/g. The bound and free MH were measured by different analytical techniques, and this discrepancy is within experimental error of the two methods.

Figure 5. Plot showing the variation of normalized MH by TPM in smoke as a function of free MH in 1 g tobacco

Figure 6. Plot showing the variation of normalized MH by TPM in smoke as a function of total MH in 1 g tobacco

amount of MH in smoke by the amount of total particulate matter (TPM), and by calculating the total and free levels of MH for 1 g of tobacco. The results are summarized in Table 3 and were used to produce Figures 5, 6, and 7.

The first finding in this study can be obtained by inspecting the graphs that show the variation of MH in smoke normalized by TPM levels as a function of the amount of MH per 1 g tobacco. The graph for free MH in tobacco is shown in Figure 5 and the graph for total MH in tobacco is shown in Figure 6. As seen by comparing the two dependencies, a much better correlation is obtained for the dependence of MH in smoke as a function of total MH rather than free MH, in 1 g tobacco (R^2 coefficient 0.93 for total MH vs. R^2 coefficient 0.66 for free MH). This result indicates that the MH in smoke depends on the total MH present in tobacco, and not only on the free MH.

Further evaluations were done to establish the rate of transfer of MH to smoke, considering the total amount of MH in tobacco. Attempting a non-linear correlation between the amount of MH in tobacco and the amount of

MH normalized by TPM in smoke, it can be seen that the relative transfer rate decreases as the level of MH increases. This is shown in Figure 7 where the slope of the curve decreases at higher MH levels in tobacco. The increase in the transfer rate of MH at the lower levels can be explained by the hypothesis that MH is formed in smoke even when it is absent in tobacco, as reported by LIU *et al.* (6). This formation appears to be quite unlikely considering the chemical structure of MH. However, the analysis of tobacco for the presence of total MH is relatively difficult. Part of the bound MH in tobacco may remain unhydrolyzed during analysis and therefore undetected. This undetected MH in the tobacco may be the cause of the apparent formation of MH in the smoke of cigarettes with zero values for the analytical MH level. If this is the case, the MH formed from the bound undetected MH has a greater impact on the transfer rate calculation for low MH-containing tobaccos than from high MH-containing tobaccos. This hypothesis was not verified experimentally in the present study.

Figure 7. The estimation of transfer rate of MH/mg TPM for various levels of total MH in 1 g tobacco

Limiting the level of MH in tobacco to about 40 µg/g tobacco, the transfer rate is about 0.24%/mg TPM, but it can range as high as 0.46%/mg TPM for a nonfilter cigarette low in MH. Lower transfer rates are seen for higher levels of total MH in cigarette, the values being around 0.14%/mg TPM.

As seen from these results, the amount of MH in smoke is a function of cigarette TPM, and higher TPM levels lead to more MH in smoke. By reporting the results as MH/mg TPM, it was attempted to eliminate factors related to cigarette construction (tobacco weight, cigarette paper porosity, ventilation, filter efficiency). The results expressed as MH/mg TPM for 1 g tobacco can be easily expressed as transfer rate for a particular cigarette. For a nonfilter low MH level cigarette the transfer is about 8.3% from the total MH in the cigarette. Lower transfers are seen for other cigarettes.

CONCLUSIONS

This report presents the results of MH measurements in tobacco and in cigarette mainstream smoke. The amount of MH in smoke is a function of cigarette TPM, and higher TPM levels lead to more MH in smoke. The transferred level of MH depends on total amount of MH (both bound and free) and not only on the free MH level in tobacco. The relative transfer is higher for lower tobacco MH levels than for higher MH levels. Regardless of the cigarette construction, the transfer is about 0.24% per mg TPM as an average. However, for a nonfilter cigarette low in MH, as much as 0.46%/mg TPM is transferred from 1 g tobacco. This is equivalent to 8.3% from the total MH in the cigarette. The findings are in agreement with those of LIU and HOFFMANN (2).

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