Exploring the ‘Rolling Unmasking Effect’ of Downwind Odor Dispersion from Model Plant and Animal Sources

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Abstract: Solving environmental odor issues can be confounded by many analytical, technological, and socioeconomic factors. Considerable know-how and technologies can fail to properly identify odorants responsible for the downwind nuisance odor and mitigate it for the affected citizenry. We propose enabling solutions to environmental odor issues by utilizing troubleshooting techniques developed for the food, beverage, and consumer products industries. We showed that the downwind odorant impact-priority ranking process can be definable and relatively simple. The initial challenge is the prioritization of environmental odor character from the perspective of the impacted citizenry downwind. In this research, we aim at summarizing three natural models of the rolling unmasking effect (RUE) and discuss them more systematically in the context of the proposed downwind environmental odor prioritization approach. Regardless of the size and reach of an odor source, a simplification of odor character and composition typically develops with downwind dilution. The extreme odor simplification-upon-dilution was demonstrated for two plant varieties, prairie verbena and Virginia pepperweed. Their downwind odor frontal boundaries were dominated by single, character-defining odorants; p-cresol-dominated ‘barnyard’ odor, and benzyl mercaptan-dominated ‘burnt match’ odor, respectively. The P.T. porcupine downwind odor frontal boundary was dominated by two potent, character-defining odorants: (1) ‘onion’/‘body odor’ odorant #1 and (2) ‘onion’/‘grilled’ odorant #2. In contrast with their downwind boundary simplicities, each odor source presented considerable compositional complexity and composite character difference near the source. The proposed RUE approach’s ultimate significance is the illustration of naturally occurring phenomena that explain why some environmental odors and their sources can be challenging to identify and mitigate using the analytical only approach (focused on compound identities and concentrations). These approaches rarely move beyond comprehensive lists of compounds being emitted by the source.

Keywords: odor, volatile organic compounds, environmental analysis, air sampling, simultaneous chemical and sensory analysis, prairie verbena, prehensile-tailed porcupine, Virginia pepperweed.

1. Introduction

1.1 Is solving the downwind odor problem possible without an exhaustive list of identified compounds emitted from a source?

Concerning contemporary environmental odor issues, odorant prioritization does not appear widely recognized or referenced. It is still common to refer to an odor issue as approximately correlated to an extensive inventory listing of volatile chemicals that are shown to be emitting from a ‘suspect’ odor source. Sometimes the listing represents an extensive and complex emission mixture; potentially encompassing hundreds of VOCs
and many chemical functionalities. For illustrative examples, lists of volatile organic compounds (VOCs) distributed between (a) organic sulfides; (b) ketones; (c) aliphatic aldehydes; (d) aromatic aldehydes; (e) aromatic hydrocarbons; (e) terpenes; (f) alcohols; (g) volatile fatty acids; (h) hydrocarbons; (i) chlorinated hydrocarbons and (j) aliphatic siloxanes as in the case of odor emissions from sewage treatment facilities [1].

Unfortunately, these extensive lists often include a preponderance of compounds with little, if any, downwind odor impact beyond the source fence-line. Also, these extensive inventories often fail to include or identify the specific odorant or odorants, which are primarily responsible for the downwind odor. In one notable example from an odorant prioritization study to the rendering industry [2], just two odorants (trimethylamine (TAM) and dimethylsulfide (DMS)) were identified as the impact-priority odorants downwind of a fish meal processing plant. This finding stands in marked contrast to an earlier study of the same issue [3], reporting ~300 organic compounds, 40 of which were odorous and stating that ‘odorous compounds included alkanes, alkenes, ketones, hydrocarbons, alcohols, alkyl halides, fatty acids, amines, aromatics, aldehydes, and epoxides.’ It should be pointed out that this 300-compound listing did include TMA and DMS, but those were not prioritized.

1.2 Challenges to the current state of the art in downwind odor assessment

Environmental odor issues can be confounded by many analytical, technological, and socioeconomic factors. While considerable know-how and technologies exist for industrial source odor mitigation, they are often not adapted for rural and agricultural odor [4]. Source-to-receptor separation can aid in lowering the risk of downwind odor impact. The dispersion has historically been described as ‘downwind dilution’ and monitored by standard techniques such as dynamic dilution olfactometry [5-7]. Still, odor sample loss problems have been identified [8,9].

There is also broad recognition of a challenge to link specific compounds to resulting downwind odor [10,11]. The odor activity value (OAV) concept has been used with some success to show that compound-specific odor detection thresholds (DTs) can be helpful to explain why some compounds are more impactful odorants than others [12-15]. The use of simultaneous chemical and sensory analyses has also gained acceptance as a technology for isolating, ranking, and prioritizing odor-causing compounds in a complex mixture of gases [16-19]. Downwind odor dilution, while helpful, can also limit resolving environmental odor impact issues [20,21]. Our experience has shown that the odor ‘character’ (defined as a descriptor of what it smells like) from an environmental source can depend upon the downwind distance from that source, potentially radically different nearest the source relative to locations farther removed [20-24].

1.3 Proposed solution – simplification of odorant prioritization downwind

We propose solving environmental odor issues by utilizing troubleshooting techniques developed for the food, beverage, and consumer products industries. Our experience has shown that an odorant impact-priority ranking is definable for virtually every odor source, whether natural or human-made. The initial challenge is the prioritization of environmental odor character from the perspective of the impacted citizenry downwind.

With increasing distance separation from the source, the process of downwind environmental odorant prioritization can be described as a rolling unmasking effect (RUE) Figure 1. The RUE is exhibited by the odors nearest the source sequentially ‘falling away’ with distance from the source, revealing (unmasking) a succession of increasingly simplified odor composition and character. The RUE ends at the downwind odor frontal boundary with an odor character dominated by the impact-priority odorant (or a handful of odorants). These are the odorant(s) with the greatest downwind sensory reach (impact). For example, this has been shown for p-cresol, as a ‘signature’ downwind odor from confined animal feeding operations (CAFOs); recognizable at a great distance from the source [22,23], in one case remaining the single, most offensive characteristic compound as far as 16 km away [24].
Figure 1. Pictorial representation of ‘rolling unmasking effect’ (RUE). The source is a complex mixture of odorants, yet it is simplified to a handful of impactful odorants (illustrated with the yellow dot) at the receptor. The odor frontal boundary represents the farthest downwind reach (impact), while the internal bands represent the points of sequential odor unmasking as the secondary-impact odorants are diluted below their detection/masking concentration levels.

1.4 Relying on sensory experience for recognizing the simplification to odorant prioritization downwind

Without conscious effort the impacted citizenry can associate some characteristic environmental odors and specific chemicals primarily responsible for those odors. For example, a parent’s recognition of ammonia as the specific chemical most responsible for the odor near an incubating pile of urine-soaked diapers is a simple manifestation of that innate ability. The primary factor separating the citizenry from professionals, tasked with deconstructing the chemical composition of diverse odors is the number and obscurity of such chemical-smell associations, which can be made in advance of analytical confirmation.

1.5 Examples of RUE

Several examples of the RUE have been described over the past two decades. Examples are (1) odor from the large colony of Mexican free-tailed bats (i.e., *Tadarida brasiliensis*) [20,21,25] and (2) odor from a large cattle feedlot [22,23]. For the Mexican free-tailed bat colony [25], three distinct odor boundaries (Figure 1) were definable relative to the cave source: (i) an overpowering ‘ammonia’ odor within the cave and for ~15 m downwind of cave openings; (ii) emergence of a composite ‘rat nest’ odor, which was dominated by 4-methyl quinazoline upon the decline of the masking by ammonia, and (iii) emergence of the characteristic ‘bat cave,’ ‘taco shell’ odor, dominated by 2-aminoacetophenone, upon approach to the outer ‘odor frontal boundary’; enabled by the decline of odor masking by 4-methyl quinazoline. Similarly, for the feedlot source [22,23], at least two distinct odor boundaries were definable (i) a strong ‘fishy’/‘amine’ odor dominated by TMA within the feedlot and for several hundred meters downwind, and (ii) the emergence of a ‘barnyard’ odor, dominated by p-cresol, upon approach to the outer ‘odor frontal boundary’; enabled by the associated decline of downwind odor masking by TMA.

1.6 Objective
We aim at summarizing three new examples of RUE and discuss them more systematically in the context of the proposed downwind odor prioritization approach. These new RUE examples involve animal and plant-related odor. This research focused on three contrasting RUE examples:

- Prairie verbena (Verbenaceae Glandularia bipinnatifida),
- The South American prehensile-tailed porcupine (Coendou prehensilis), P.T. porcupine
- Virginia pepperweed (Brassicaceae Lepidium virginicum).

The ultimate significance of the proposed RUE approach is the illustration of naturally occurring phenomena that explain why some environmental odors and their sources are challenging to identify and mitigate using the analytical-only approach (focused on compound identity and concentration) and rarely moving beyond comprehensive lists of compounds emitted by the source.

1.7 Rationale

The RUE process is the same regardless of the source and reach. In each case the sources’ VOC profile is often highly complex. For natural sources, this complexity is often reflected in hundreds of discrete VOCs, dozens of which are likely odorous and, therefore, carry the potential for odor-impact significance, downwind. The natural dilution process simplifies the at-source odor complexity with increasing distance separation. The source-to-frontal odor boundary’ RUE often results in emergence of a simplified subset of the priority odorants. Simplification can be reflected in the subset composition and the total number of odorants essential for inclusion. The logistics involved in carrying out an odorant prioritization can be challenging when targeting large area odor sources (e.g., industrial or CAFO) due to the size and the downwind impact. However, natural source models herein illustrate significant reductions in size and distance of their downwind reach while explaining how the RUE approach could be adopted for solving larger scale odor issues.

2. Materials and Methods

2.1 Multidimensional Gas Chromatography-Mass Spectrometry-Olfactometry

Simultaneous chemical and sensory analyses combined olfactometry (O), multidimensional (MD) separation techniques with conventional GC-MS instrumentation. An MDGC-MS-O system was used for odorant prioritization. The system consisted of an Agilent 6890 GC/5975B MS modified for MDGC-MS-O utilizing an AromaTrax control system (Volatile Analysis Corp., Round Rock, TX). Details regarding general hardware and operation have been described elsewhere [26,27]. Specific operational parameters were: injection mode: split-less with solid-phase microextraction (SPME) sample collection and delivery; injection temperature: 250 °C; detector #1: FID (280 °C); detector #2: Agilent 5975B MSD in MS-SCAN or SIM acquisition modes; column #1: 12 m x 0.53 mm ID BPX 5 - 1.0 µm film (pre-column from SGE); column #2: 25 m x 0.53 mm ID BPX 20 - 1.0 µm film (analytical column from SGE); column temperature program (overview survey and MDGC-MS-O): 40 °C initial, 3 min hold, 7 °C·min⁻¹, 220 °C final, 20 min hold.

2.2 MDGC parameters for compound isolation with heart-cutting

Concerning MDGC heart-cut isolation/clean-up of the two target ‘onion’ odorants for the P.T. porcupine: (1) optimal band for heart-cut #1 (i.e., unknown ‘onion’ odorant #1) was ~ 9.9 to 11.2 min; (2) optimal band for cryotrap #1 was ~9.4 to 11.5 min; (3) optimal band for heart-cut #2 (i.e., unknown ‘onion’ odorant #2) was ~14.4 to 15.8 min; (4) optimal band for cryotrap #2 was ~13.9 to 16.1 min; (5) long SPME collection of the whole urine headspace yielded overwhelming odor responses but no obvious associated mass spectral ion detail for the critical ‘onion’ odorants. In contrast to the MDGC based heart-cut isolation protocol, as applied to the ‘onion’ odorants for the P.T. porcupine urine, both the prairie verbena and Virginia pepperweed headspace VOC / odor profiles were processed in overview survey mode; with total heart-cuts taken between 0.25 min to 32.0 min.

2.3 Chemical Identification
The MS was operated in MS-SCAN mode for survey mode odorant identification. The mass range (35 to 400 amu) was scanned at 3.84 scan s⁻¹. The resulting spectra were analyzed with Benchtop PBM software, referencing the Wiley 7 library for the best-match ranking against the database. The panelist retained final over-ride determination as to the likelihood of correctness of the best-match listings. Spectra without a suitable library match were considered ‘unknown’; unless overridden by considerations of known retention time combined with simultaneous odor character recognition at the sniff port.

The proposed character-defining odorant identities for both the prairie verbena and Virginia pepperweed were confirmed via GC retention time and odor character matching. Unfortunately, the panelist failed to confirm the two character-defining ‘grilled onion’ chemical identities from the P.T. porcupine. Therefore, in a further attempt to identify these unknowns, collaborations with experts in the food flavor/aroma field were engaged (†T.H.; ‡A.I.). Their approaches are summarized in detail in the Supplementary Materials. Briefly, collaborator †1 used purge-and-trap thermal desorption followed by GC-MS-based analyses. Collaborator ‡2 used the same MDGC-MS-O approach as the panelist and served as cross-checks of the proposed VOC / odorant identity profiles.

2.4 PT porcupine urine sampling

A urine sample with the entrained fecal matter was collected (see details in Supplementary Materials). The VOCs were collected from the equilibrated headspace formed within a 1-quart glass headspace vessel containing a few drops of the urine sample, injected onto a crumpled low-odor paper towel substrate. The sample was equilibrated, stored, and sampled in an open-air laboratory environment at 24 °C. In addition, direct comparison samples were collected utilizing a single, designated, 1 cm × 75 µm Carboxen/PDMS SPME fiber (Supelco, Bellefonte, PA). SPME fiber insertion into the headspace was through a pinhole placed in the vessel’s PTFE closure. The amount of extracted VOCs was varied by altering the time the SPME fiber was exposed to the equilibrated headspace.

2.5 PT porcupine exhibit - downwind air sample collections with SPME

A series of direct environmental air samples were collected with SPME. The SPME fibers were: (1) preconditioned at 260 °C; (2) transported, under dry-ice storage conditions, to the Moody Gardens Rainforest for the VOC collection by direct SPME fiber exposure within the P.T. porcupine indoor exhibit and (3) return of samples to the laboratory under dry-ice storage conditions. Preconditioned SPME samplers were secured onto a field-support fixture within the exhibit enclosure; the adsorbent coated fiber tips extended from their protective needle sheaths (i.e., exposed to the enclosure environment). SPME exposures to air were executed for 7 and 9 min, respectively. Duplicate SPME fibers were exposed for 15 h. Finally, the four sample collections were transported, under dry-ice conditions, back to the laboratory for odorant prioritization assessment. At the time of sample collection, the smell, far downwind from the enclosure, was described as distinct ‘grilled onion’.

2.6 Prairie Verbena

The preconditioned 1 L glass headspace vessels were filled with freshly harvested blossoms, collected at peak maturity from a dense natural cluster (Georgetown, TX). At the time of harvesting, the smell at the odor frontal boundary was characterized as a distinct ‘barnyard’, identical to that recalled for the pure odorant, p-cresol.

2.7 Virginia pepperweed

The preconditioned 1 L glass headspace vessels were filled with freshly harvested whole plants at peak maturity (i.e., stems + blossoms), reflecting either ‘pristine’ or ‘crushed’ conditions. Crushing was necessary to release the characteristic ‘burnt match’ odor. The plants were harvested from sparse/random distribution within lawn/field environments (Georgetown, TX). When harvesting, the macerated whole plants’ smell at the frontal boundary was characterized as a distinct ‘burnt match,’ identical to that recalled for the pure odorant benzyl mercaptan.
3. Results

The model case studies below serve as an illustration of the RUE concept. These cases document an apparent (i) difference between the overall odor perceived at the source and the downwind receptor, accompanied by (ii) chemical and odor simplification. The ultimate goal is to use RUE to correlate the downwind odor with the individual chemical(s) most responsible for that odor.

3.1 Case Study #1: Prairie Verbena

Prairie verbena varieties have a wide range of odor characteristics spanning from virtually odorless (i.e., especially among many commercial hybrids), pleasantly fragrant, to remarkably unpleasant. The focus of this case study is the prairie verbena (i.e., Verbenaceae, Glandularia bipinnatifida); specifically, the regionally isolated variety of verbena, which is native to the boundary region between adjacent Blackland Prairie and Edwards Plateau ecological regions near Georgetown, Texas.

3.1.1 Initial odor assessment at the source and downwind

Upon entering the verbena’s odor frontal boundary, the panelist was immediately struck by its surprising intensity of familiar ‘barnyard’ odor (perceived as similar to past studies targeting mammals as environmental odor sources [5,22,23]). After eliminating obvious CAFO as a source, a closer inspection of the prairie verbena clusters upwind was confirmed as the ‘barnyard’ odor source (Figure 2).

![Image](https://via.placeholder.com/150)

**Figure 2.** Prairie verbena source of ‘barnyard’ downwind odor. (1) Wind direction; (2) ‘barnyard’ odor frontal boundary; (3) ‘floral’ secondary (near-source) boundary; (4) investigator’s location upon initial encounter in Georgetown, TX and (5) location of odor source cluster of native prairie verbena. Google Earth image.

The working hypothesis was: ‘due to the remarkable similarity in downwind odor characteristics, there is assumed to be some chemical compound commonality between the priority odorant subsets for the prairie verbena and typical ‘agrarian,’ sources of ‘barnyard’ odors.’ The driving questions were:

- Are there common character-impact odorants for both prairie verbena and swine CAFO sources that account for the striking similarity in composite odor at their respective odor frontal boundaries?
What is the overall agreement between the prairie verbena and swine CAFOs when comparing their minimum priority odorant subsets downwind and their full (at source) odorant and underlying VOC profiles?

3.1.2 Odorant Prioritization

The analytical phase focused on the complex VOC emissions (Figure 3, total ion chromatogram) and corresponding odorant profile (Figure 4, aromagram). The volatiles profile (Figure 3) approached 100 discrete compounds even though the initial sampling and sample preparation favored relatively low extraction. As a result, the smallest chromatographic peaks represent concentrations in the high ppt to low ppb range. The complexity is also reflected in the range of chemical functionalities, including terpenes, hydrocarbons (saturated and unsaturated), ketones (aliphatic and aromatic), alcohols (aliphatic and aromatic), esters (aliphatic and aromatic), and phenolics. Likewise, the odorant profile (Figure 4) approaches 50 discrete odor ‘notes’.

Figure 3. Overview of the prairie verbena headspace volatiles; TIC overview VOC profile, generated in ms-SCAN acquisition mode.
Figure 4. Overview odor profile of the prairie verbena headspace volatiles; aromagram odor profile, generated by a trained panelist evaluating separated odor ‘notes’ at the sniff port of MD-GC-MS-O.

These ‘modest’ VOC (~100 peaks in Figure 3) and odor note (~50 notes in Figure 4) numbers are certain to increase several-fold if pre-concentration is required to reduce the detection limits from the low ppb to low ppt and below. Such trace-level concentrations are odor-significant for many odorous VOCs. Without direct sensory correlation (Figure 4), the analyst taking the instrument-only approach (Figure 3) is faced with the daunting task of identifying all possible odorants representing potential high-impact and inferring, which ultimately could constitute a character-impact subset. Instrument-only efforts often end in disappointment since the highest-impact odorants are typically at trace concentration levels, often ‘buried’ within a complex background matrix of chromatographic peaks.

Likewise, the lack of critical compositional data (Figure 3) can derail the sensory-only approach at the opposite extreme. Interestingly, among the arguments used for necessitating a sensory-only approach is the analytical complexity. It is often argued that the human olfactory response is inherently complex and, as a result, the composite odor from such complex mixtures is inevitably the ‘combined effect’ of many, if not most, of the odorous VOCs making up the complex emission field. Thus, it might be argued that the characteristic ‘barnyard’ odor, perceived downwind of the prairie verbena source, is the combined response to the ~50 odorants (Figure 4). However, the MDGC-GC-O-based odorant prioritization indicates that this was not the case.

The characteristic ‘barnyard’ odor downwind was perceived as relatively simple; primarily traceable to a single odorant (p-cresol). Simplification was achieved using a series of odorant prioritization runs, using SPME exposure time variation to emulate a serial dilution process (i.e., from 70 min ‘undiluted’ reference, 15 min, 5 min, 102 s, 33 s, 11 s, to 3 s). Contrasting VOC / aroma profiles, comparing the ‘dilution’ steps, are shown in Figure 5 (15 min vs. 102 s), Figure 6 (102 vs. 11 s), and Figure 7 (15 min vs. 11 s).
Figure 5. Serial dilution comparisons of the prairie verbena headspace volatiles; TIC VOC profiles, generated in ms-SCAN acquisition mode. Contrasting volatiles collections of 15 min (bottom) and 102 s (top) SPME fiber exposure times. TIC chromatograms are displayed in mirror-image format. 

*p*-Cresol emerges as one of the key odorants.

Figure 6. Serial dilution comparisons of the prairie verbena headspace volatiles; TIC VOC profiles, generated in ms-SCAN acquisition mode. Contrasting volatiles collections of 102 s (bottom) and 11 s (top) SPME fiber exposure times. TIC chromatograms are displayed in mirror-image format. 

*p*-Cresol emerges as the key odorant.
Figure 7. Serial dilution comparisons of the prairie verbena headspace odorants; aromagram odor profiles, generated by GC-Olfactometry. Contrasting odorant collections of 15 min (top) and 11 s (bottom) SPME fiber exposure times. Aromagrams are displayed in mirror-image format and illustrate how the p-cresol emerged as the key odorant.

The odorant prioritization suggests that the ‘barnyard’ odor downwind is carried almost exclusively by the single, character-defining odorant, p-cresol. Key supporting evidence for this conclusion is:

- (1) the ‘barnyard’ odor character, as perceived at the MDGC-MS-O sniff port for the isolated p-cresol peak, was virtually identical to the ‘barnyard’ smell perceived by the panelist at the odor frontal boundary (Figure 2);
- (2) the p-cresol peak was the last detectable odorant response under the maximum dilution (i.e., 3 s SPME fiber exposure);
- (3) the response to p-cresol as ‘barnyard’ was the only and last detectable odorant, despite the extremely brief, 3 s, exposure period, a condition known in SPME to bias against semi-VOCs.

Simplification was also reflected in an ~10-fold reduction in the VOC number and a corresponding 4-fold reduction in odorant ‘notes’ (Figures 5-7), a process helpful in developing a simpler synthetic odorant subset (Table S1, Supplementary Material). The odorant priority subset was narrowed to:

- p-cresol (character-defining ‘barnyard’ or ‘hog-truck’ at the odor frontal boundary);
- –oxime isomers @6.0 min R.T. with their ‘ether’ or ‘ketone’ odor character;
- trans-calamanene @24.2 min with its ‘spicy’ ‘baked bean’ odor character;
- alpha-pinene with its ‘pine oil’ odor character;
- hyacinthin @18.0 min R.T. with its ‘floral’ odor character, and
- 1-octene-3-one with its ‘earthy’ or ‘mushroom’ odor character.

This evidence would be sufficiently conclusive to justify proceeding with subsequent validation and instrument-based monitoring protocol development if this investigation had been tied to an actual environmental odor issue. These follow-up efforts would focus on p-cresol as the target ‘character-defining’ odorant for prioritizing odor monitoring and
mitigation assessment purposes. Supplementary Material provides an additional interpretive process for balancing the six-component priority subset with the secondary and tertiary priority odorants.

3.2 Case Study #2: Prehensile-tailed Porcupine

3.2.1 Initial odor assessment at the source and downwind

The smell encountered at the downwind odor frontal boundary (Figure 8) reflected a very distinct and familiar ‘grilled onion’ and ‘1950s hamburger joint’ character. The panelist walked upwind to determine where the food court must be located. However, upon walking deeper into the odor plume, the panelist encountered, almost simultaneously, an intense ‘foul’ odor and an associated exhibit display sign which read, ‘What is that Foul Odor?’ The sign heading was followed by a description of the P.T. porcupine exhibit as the source.

Figure 8. P.T. porcupine encounter in Moody Gardens. (1) Wind direction; (2) odor frontal boundary; (3) approximate secondary (near-source) boundary; (4) investigator’s approximate location upon initial encounter and (5) location of outdoor enclosure of the odor source the P.T. porcupine. Google Earth image.

The near-source smell was perceived as ‘phenolic,’ ‘industrial,’ and ‘foul.’ The dramatic difference in character was particularly surprising considering that only a few paces separated the pleasant ‘grilled onion’ at the odor frontal boundary and the ‘foul’ deeper into the plume.

The working hypothesis was: ‘due to the remarkable similarity in downwind odor characteristics, there is assumed to be some chemical compound commonality between the priority odorant subsets for the P.T. porcupine and typical ‘grilled onion’ odors. The driving questions were:

- Are there common character-impact odorants to both the P.T. porcupine and typical ‘grilled onion’ sources that account for the striking similarity in composite odor at their respective odor frontal boundaries?
• What is the overall agreement between the P.T. porcupine and typical ‘grilled onion’ when comparing their minimum priority odorant subsets downwind and their full (at source) odorant and underlying VOC profiles?

3.2.2 Odorant Prioritization

The MDGC-MS-O-based odorant prioritization confirmed the pre-analysis assumption that the impact-priority odorant would be found to be traceable to a specific homolog from the extensive ‘onion’ odor allylic-polysulfide family [29, 30]. The ‘1950s hamburger joint’ odor note had previously been isolated and described (i.e., by retention time and ‘sniff port’ detector basis only) in prior onion-sourced odorant prioritization investigations. Upon inspection of P.T. porcupine urine headspace, the ‘grilled onion’ odor note eluted a few seconds before dipropyl trisulfide and earlier still than the propyl – propenyl trisulfide isomer series @20.8 min. A second ‘onion’ note (i.e., unknown ‘body odor’, ‘onion’ @13.9 min) was also found. Remarkably, the extremely complex headspace odor profile appeared free of other members from the onion-sourced allylic-polysulfide family (Figures 9 and 10).

Figure 9. MS-SCAN TIC chromatogram of the P.T. porcupine indoor exhibit chamber VOCs; total ion overview VOC profile, generated in ms-SCAN acquisition mode. Volatiles collection by 15 h SPME fiber exposure to the chamber environment.
These two character-defining ‘onion’ odorants were shown to emerge from a vast and complex odorous VOC field common to mammalian waste [31-34]. The chemical identification effort for the two character-defining, ‘onion’ odor compounds included three alternative approaches. Despite these considerable efforts, the identifications of the two, character-defining, ‘onion’ odors remained elusive. The likely reason is the extreme trace concentration and odor potencies of these two odorants. In addition, work to date suggests that the targeted unknown ‘onion’ carrier compounds are not related to specific polysulfide odorants, previously reported as responsible for ‘grilled onion’ and ‘fried onion’ odor character [35-37].
Figure 12. ms-TIC Chromatogram of male P.T. porcupine urine headspace VOCs; analytical column separation of two ‘onion’ carrier target heart-cut isolation bands (cryo-trapped). Volatiles collection by 60 min SPME fiber exposure.

Figure 13. Total Ion Chromatogram of male P.T. porcupine urine headspace VOCs; analytical column separation focused on the second of two cryo-trapped ‘onion’ carrier target heart-cut isolation bands. Volatiles collection by 10 min SPME fiber exposure.

3.2.3 Contrasting downwind odorant prioritizations - the P.T. porcupine vs. a swine-barn:

The VOC emission composition for P.T. porcupine and swine barn have much in common (Table S2). The P.T. porcupine, like the CAFOs, presents with significant emission loadings of the reduced sulfurs, free-fatty acids, indolics, and phenolics (i.e., including p-cresol), all of which factor heavily in CAFO emission profiles. The absence of CAFO-like odor character from these odorous VOCs at the P.T. porcupine’s odor frontal boundary magnifies the impact significance of the two unknown ‘onion’ odorants as the characteristic ‘grilled onion’ odor carrier. It is particularly interesting that the P.T. porcupine and
swine barn sources generate distinctly different odor characteristics at their respective odor frontal boundaries; despite sharing much in common through their VOC emission profiles at the source.

3.3 Case Study #3: Virginia pepperweed

3.3.1 Initial odor assessment at the source and downwind

The first conscious introduction of the panelist to the smell was while mowing a lawn with small clusters of Virginia pepperweed. At the source, the overall smell had the expected ‘freshly cut grass’, ‘earthy’ character. However, when the downwind odor frontal boundary was approached, a very familiar, intense, and distinct ‘burnt match’ odor character was detected. This ‘burnt match’ odor was recognizable due to past studies targeting various environmental odor sources [38].

The working hypothesis was: ‘due to the remarkable similarity in downwind odor characteristics, there is assumed to be some chemical compound commonality between the priority odorant subsets for the Virginia pepperweed and some ‘burnt match’ odors. The driving questions were:

- Is benzyl mercaptan correctly predicted as the character-defining odorant responsible for the ‘burnt match’ smell at the time of that first encounter and in advance of analytical confirmation?
- What is the general agreement between the Virginia pepperweed and recognized ‘burnt match’ odor sources when comparing their minimum priority odorant subsets downwind and their full (at source) odorant and underlying VOC profiles?

3.3.2 Odorant Prioritization

Follow-up MDGC-MS-O based odorant prioritization efforts confirmed the pre-analysis prediction that the character-defining odorant was traceable, dominantly to benzyl mercaptan (Figure 14). This prediction was significant; considering, that the panelist had to recognize and correctly identify a single odorous chemical from the hundreds, or perhaps thousands, of other possible odorous chemicals. The mechanical stressing of the plant was critical to release the odorous VOCs, as shown in the contrasting ms-SCAN TIC chromatograms profiling pristine versus crushed emission profiles.

![Figure 14. ms-SCAN TIC chromatogram of the Virginia pepperweed headspace volatiles. Total ion overview profiles are displayed in mirror-image format, reflecting pristine versus crushed states.](image)

The overall odorous VOC emission profile from the crushed plant was complex, including prominent peaks of (1) benzyl thiocyanate; (2) benzyl isothiocyanate; (3) hexanal;
(4) 3-hexene-1-ol; (5) benzaldehyde, and (6) benzene acetonitrile, among others (Figure 14). Interestingly, the character-defining benzyl mercaptan peak was not detectable under the initial ms-SCAN survey acquisition parameters. Therefore, the chemical identity confirmation for benzyl mercaptan was enabled by matching the known chromatographic retention time and known distinctive odor response for benzyl mercaptan at the olfactory detector. The corresponding odor profiles for the pristine versus crushed are shown in Figure 15.

Figure 15. Comparison of odor profile aromagrams of the crushed and pristine Virginia pepperweed.

The apparent simplicity of the benzyl mercaptan as ‘impact-priority’, responsible for the ‘burnt match’ odor at the frontal boundary, progressed to a distinctly different odor, of much greater complexity at the source. The overall odor near the opening of the 1 L glass vessel containing the crushed plants was perceived as ‘grassy’, ‘herbaceous’, and ‘earthy’/‘mushroom’. The overall impact-priority subset for the crushed Virginia pepperweed source was therefore proposed as (1) benzyl mercaptan (character-defining ‘burnt-match’ at the odor frontal boundary); (2) hexanal ‘grassy’; (3) 1-octene-3-one ‘earthy’; (4) 3-hexene-1-ol ‘herbaceous’; (5) unknown ‘ketone’ @13.59 min R.T.; (6) benzaldehyde ‘cherry’; (7) unknown ‘burnt’ @24.95 min R.T.; (8) methyl mercaptan ‘fecal’ and (9) hydrogen sulfide ‘sewer’.

4. Discussion

4.1 Implications of the Rolling Unmasking Effect and Odorant Prioritization for Environmental Odor Mitigation and Monitoring Strategy Development

These results illustrate an important consideration and a model for larger-scale environmental odor sources. To date, the source is often the focus when challenged with solving a downwind odor issue. While it is essential to do so, with respect to downwind odor impact it is possible to ‘look’ too closely at the source. Focusing on all compounds present at the source often expands the study to include background noise, an unnecessary expenditure if the goal is to reduce downwind environmental odor impact.

It is recommended to initially focus on the smallest subset of odorous chemicals, representing significant impact and reach downwind. This simplification was illustrated by (1) the benzyl mercaptan (responsible for the ‘burnt match’ odor of Virginia pepperweed);
(2) the two unknown ‘onion’ odorants responsible for the ‘grilled onion’ odor of the P.T. porcupine and (3) the p-cresol responsible for the ‘barnyard’ odor of prairie verbena. These character-defining odorants are first recognizable at the odor frontal boundaries. The remaining complex odorous ‘noise’ near the source is often eliminated through the natural dilution process downwind. Success in mitigating the highest impact odorants results in moving the odor frontal boundary back toward the source, reducing its downwind reach (i.e., the most efficient approach to developing effective remediation, monitoring, and mitigation strategies).

The actual reduction in downwind reach could be realized by selective elimination of only those few character-defining compounds. However, as was shown in Figure 1, the reduction in downwind odor reach will actually be determined by the distance separation between the outermost frontal boundary and the nearest secondary boundary in retreating upwind toward the source. With regard to such a ‘hypothetical’ selective elimination strategy, the best-case scenario would be a considerable distance separation between the frontal boundary and the closest secondary boundary. This may or may not be what actually exists relative to a source. The natural, steady-state profile for a source could reflect a relatively narrow separation between frontal and secondary boundaries.

It is possible to encounter an unintended consequence of the selective elimination of only the (one) character-defining odorant which is responsible for the odor at the frontal boundary. The result could be the ‘unmasking’ of another odorant with odor character even more offensive than the original. An example of this is reflected in the P.T. porcupine odor. The selective elimination of only the ‘grilled onion’ character-defining odorants would elevate the rest of the impact-priority odorant subset. The emerging secondary odor boundary could be more ‘barnyard’ in character [20,21], owing to the p-cresol prominence and the overall odor profile similarity between P.T. porcupine and swine barn (summarized in Table S2). Therefore, a more realistic strategy is to mitigate the smallest character-impact subset of odorants responsible for frontal boundary and near-source odor character. For example, in the case of the P.T. porcupine, that smallest combined, impact-priority subset consists of the 5 to 7 odorants leading (Table S2).

4.2 Counter-intuitive odor masking

The impact-priority rankings can be counter-intuitive, revealing an unexpected difference in odor-defining compounds near-source vs. frontal boundary. The OAV concept has been applied to gauge the difference in odor potency between different odorous compounds emitted from a source. OAV is defined as the ratio between an odor-ant’s concentration and the odor threshold concentration of that compound. Unfortunately, the concept fails to adequately explain the apparent difference in odor dominance by compounds because it assumes to reflect relatively constant OAV values spanning the time and source-to-receptor distance (which, in practice, does not always hold).

An alternate representation of this observed counter-intuitive unmasking effect is proposed (Figure 16) based on the earlier observations [21]. The graphical representation of the odor sigmoid intensity curves for two competing odorants, reflecting relatively high vs. relatively low odor potencies; 2-aminoacetophenone and ammonia, respectively. The concentration vs. odor intensity is delineated by (1) odor threshold value - the minimal concentration that a human receptor can detect as a perceptible odor change; (2) odor recognition threshold value - the minimal concentration that can be detected and recognized by the human receptor as to odor character/odor source and (3) odor saturation threshold value - the concentration level at which all related olfactory receptors are activated and above which any additional concentration increase will fail to induce a corresponding increase in response intensity. While ammonia requires a much higher concentration to exceed the odor and recognition thresholds, once exceeded, it rises to a response level that overtakes 2-amino acetophenone. Thus, while OAV values account for the dominance of a higher-impact odorant up to being masked, it fails to account for the apparent reversal in dominance above that juncture (Figure 16). Several mechanisms have
been proposed for this observed non-linearity of the OAV values at a higher concentration, including (1) synergistic effects, (2) receptor blocking effects, (3) receptor competition effects, and possibly others.

Figure 16. Comparison of odor threshold curves for a higher impact odorant (2-amino-acetophenone, blue line, greater reach) versus lower impact odorant (ammonia, red line, shorter reach but masking) can explain why the OAV concept can fail to explain the difference between priority, odor-defining odorants downwind versus near the source.

Another example of counter-intuitive odor masking is ‘musty’ cork taint in the wine industry caused by 2,4,6-trichloroanisole (TCA) and tribromoanisole (TBA). The TCA’s ‘faint odor similar to acetophenone’, as described in the 13th Edition Merck Index [39], is noteworthy, considering its published odor threshold value of 10 parts per trillion [40]. By comparison, that for TBA is ~30 ppq [41]. The odor response to TCA or TBA contamination can be initially masked by many other common odors co-emitting from a source, regardless of relative odor potency. In contrast, however, TCA and TBA will almost always remain the ‘cork taint’, ‘musty’ defining compounds after all others have weathered away to levels below their respective odor ‘masking’ effect.

4.3 Implications of the RUE for community environmental odor issues

Understanding the RUE process can facilitate improved communication between critical stakeholders to a community environmental odor issue. Historically, the downwind citizenry have been least effectively represented in community discussions regarding odor assessment, chemical prioritization, odor monitoring, and mitigation strategy development. The communication challenges can be illustrated, at least partly, by drawing parallels from the sense of visual color perception. For example, the pictured cube (Figure 17), when presented to a human sensory panel and asked to describe the color should elicit an overwhelming response as being red. If then asked to expand on this assessment, various descriptor modifiers might be added by the panelists, such as red as ‘tomato’, ‘blood’, and ‘fire engine’. These modifiers would likely reflect a considerably lower level of consensus since they are cultural and/or personal experience-based. Fortunately, physical color-wheels can effectively neutralize these biases and reconcile the modifying descriptors to a consensus.

In contrast, for the sense of smell, we are limited solely to such subjective descriptor modifiers for reconciling communication regarding odors of common interest (e.g.,
‘sewer-like’, ‘barnyard-like’, ‘skunky’, ‘musty’). Sensory professionals representing selected industries have developed odor/aroma/flavor wheels that attempt to emulate the color wheel [42,43]. While these sensory wheels can be very effective tools in reconciling discussions between trained sensory professionals, they are too cumbersome for practical use by lay panelists (e.g., downwind citizenry). The practical challenge for such odor wheels is that they, too, rely on relatively vague descriptors such as ‘musty’, ‘barnyard’, ‘earthy’.

Figure 17. Communication about subjective odor descriptors can be more challenging than color perception.

The simplification of odor profiles, induced by the RUE, opens up the possibility of introducing a reconciling tool for odor that is more closely aligned with the simplicity of color and the color wheel. This tool uses chemical odor-matching [20,21]. For example, reconciling the communication regarding odor-character at their respective odor frontal boundaries is simplified by having a sensory panelist either confirm or reject a proposed odor-match using ‘suspect’ high-purity reference chemicals.

The odor-match query of a lay panelist relative to a targeted environmental odor can be a simple YES or NO when presented with a trace amount of a ‘suspect’ character-defining odorant. This simplicity negates the requirement for extensive panelist training, experience, or memory acuity relative to odor recognition. Such straightforward odor-match surveys can be easily expanded to include query variations such as: (1) picking the best odor-match from a multi-unknown odorant line-up, including the ‘suspect’ character-defining odorant, and (2) applying an odor-match fidelity grading estimation to a best odor-match selection. The odor-match validation process is the same whether the chemical reference is a single, character-defining odorant (e.g., dominant at the odor frontal boundary) or a multi-odorant formulation (e.g., synthetically replicating the combined frontal boundary + near-source odor character).

The odor-match approach can have some practical challenges. Even if the impact-priority odorants are isolated utilizing MDGC-MS-O, there is no guarantee that: (1) a library-match based mass spectral identification of the impact-priority subset can be achieved; (2) the suspect odorous compound(s) are commercially available for synthetic
odor-match blending or (3) the chemicals are available in sufficiently high purity (i.e., odor-purity). It is noteworthy that the two plant sources proved to be straightforward for odor-match validation, while the P.T. porcupine yielded an excellent illustration of the potential challenges. Despite extraordinary efforts utilizing: (1) MDGC-MS-O based target odorant purification/separation and (2) an 'onion' polysulfide targeted pre-concentration enrichment protocol, the identities of the two character-defining ‘onion’ odor notes remain elusive. As a result, the panelist applied the following novel concept [44, 45] for communicating regarding high-impact odorant ‘unknowns’ and ‘unavailables’.

4.4 Double-heart cut isolation of high-impact odorants from crude source materials

The novel concept utilizes MDGC, in sample-prep mode, for purification/isolation/capture of the ‘suspect’, high-purity reference odorants from readily available crude source materials. Once refined and captured, the proposed priority odorants can be utilized offline for presentation to the lay panelists for odor-match communication or validation of impact-priority or character-defining status. For P.T. porcupine, the ‘dirty’ urine was utilized as the crude source material, and the unknown ‘onion’ odorant #1 was targeted for initial odor-match validation. An inert, low-odor, polyolefin gas-tight syringe was used to ‘vacuum’ aspirate this fraction (Figure 18) capturing the targeted unknown ‘onion’ odorant #1 peak as it eluted to the olfactory detector nose-cone. The experimentally determined heart-cut effectively isolated the targeted unknown ‘onion’ odorant #1 from the bulk of potential VOC interference peaks and odorants.

![Figure 18. Whole air fraction collection process of high-impact odorants from the sniff port for offline odor assessment.](image)

Off-line composite assessment of the syringe vapor contents confirmed the odor purity of the isolated fraction and yielded consensus amongst three collaborators for the ‘onion’/‘grilled onion’ odor character descriptor. A similar consensus for the high-fidelity match to the characteristic downwind odor of the P.T. porcupine exhibit was made with the Moody Gardens Rainforest Exhibit team. However, it is also interesting to note that one team member did not characterize the odor as ‘onion’ specifically; instead, it had reminded her of a favorite sauce that her grandmother frequently made. The second team member called the odor character ‘stale onion’. These contrasting odor character descriptors reflect the need for reconciling the distinctly different contrasting descriptors, from multiple odor panelists, for the same chemical odorant.

5. Conclusions

The small scale and natural models illustrate the odorant prioritization process as an important consideration for solving community environmental odor issues. Regardless of the size and reach of an environmental odor source, a simplification of odor character and
composition typically develops with increasing downwind separation. Extreme odor simplification-upon-dilution was demonstrated for two plant varieties, prairie verbena and Virginia pepperweed. Their ‘odor frontal boundaries’ were dominated by single, character-defining odorants, p-cresol-dominated ‘barnyard’ odor, and benzyl mercaptan-dominated ‘burnt match’ odor, respectively. The P.T. porcupine downwind odor frontal boundary was dominated by two potent, character-defining odorants (1) ‘onion’/‘body odor’ odorant #1 and (2) ‘onion’/‘grilled’ odorant #2. In contrast with their boundary simplicities, each source presented considerable compositional complexity and composite odor character differences.

Although simple odor dilution occurs during downwind dispersion, the term *dynamic dilution* limits the options for solving environmental odor impact downwind. The results suggest that the process of downwind environmental odorant prioritization can better be described as a *rolling unmasking effect* (RUE). The RUE is exhibited by the masking odors nearest the source sequentially ‘falling away’ with distance from the source, revealing a succession of increasingly simplified odor characteristic and composition; reflected in the three natural model sources.

Significant parallels for community odor issues can be drawn from odorant prioritization and the RUE-driven simplification-upon-dilution:
- the potential for focusing of odor monitoring strategy development to that most technologically appropriate for the impact-priority subset of odorants,
- the focusing of odor mitigation strategy to the impact-priority subset of odorants, and
- making possible the integration of odor-matching as a reconciling tool for improving communication, among stakeholders, regarding community odor issues.

**Supplementary Materials:** The following are available online at www.mdpi.com/link, Table S1: Comparative Impact-Priority Odorants; Prairie Verbena vs. Swine Barn; Table S2: Comparative Impact-Priority Odorants; PT Porcupine vs. Swine Barn.

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