Written evidence submitted by Dr Robert D Combes and Professor Michael Balls (ECG0080)

1. Executive Summary

- As professional toxicologists interested in improving testing methods for assessing the safety to humans of chemicals, we started collaborating with the tobacco industry, to help identify promising new methods, beginning with tobacco smoking harm reduction products and then e-cigarettes.
- We soon became perplexed over the FDA’s tobacco deeming regulations and then became even more-concerned about the way in which the UK authorities were laying the foundations for using e-cigarettes in the fight against smoking-related disease. We are especially surprised by the lack of scholarship and scientific rigour that is being applied to the safety assessment of these products, and feel it important to exploit our independence by speaking out.
- The current stipulations regarding the regulatory control and authorisation of electronic cigarettes (ECs) and vaping in the UK are scientifically flawed, as they are based on little more than conjecture and value judgment, backed only by poor science.
- There has been over-reliance on chemical analysis, the use of incomplete data, and risk assessments confused with the perceived benefits of vaping versus smoking, all of which bear little resemblance to standard approaches in toxicological risk assessment.
- The authorities, and other stakeholders, have systematically ignored, or erroneously dismissed, basic principles of pharmacology and toxicology, and inconvenient scientific observations, while promoting vaping as a way of ceasing smoking, instead of discouraging the use of nicotine in any form.
- The research being overlooked includes evidence of the many pleiotropic adverse biological effects of nicotine, more of which continue to be revealed with increasing frequency, which are likely to be highly relevant to carcinogenicity and disease.
- We discuss this very serious situation, and offer some suggestions for a better way forward, for the benefit of individual humans, now and in the future.

2. Introduction

Electronic cigarettes (e-cigarettes; ECs) are handheld, electronic devices that vaporise a liquid (e-liquid) containing nicotine with other additives (e.g. propylene glycol or glycerol, and flavouring agents), and deliver the vapour to the lungs via inspiration and inhalation (a process called vaping).

In August 2015, Public Health England (PHE) declared that, in principle, ECs should be made available on prescription to reduce tobacco smoking (https://www.theguardian.com/society/2015/aug/19/public-health-england-e-cigarettes-safer-than-smoking). It was also made clear that ECs will be regulated as new medicines by the Medicines and Healthcare products Regulatory Agency (MHRA). This was followed by the news of the first e-cigarette (Evoke) to receive marketing authorisation from the agency.

These announcements have proved to be highly controversial, especially since they were justified by an estimate of there being 95% less harm from vaping than from tobacco smoking (https://www.gov.uk/government/news/e-cigarettes-around-95-less-harmful-than-tobacco-estimates-landmark-review). This submission explains why we believe that the decision by PHE is, in the light of current knowledge, irresponsible and unacceptable. We
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also propose some recommendations to avoiding the potentially very serious consequences, if this situation is allowed to continue.

3 Ignoring basic principles of toxicology

This is the most common characteristic exhibited by individuals, reports and publications discussing safety issues relating to ECs (Table 1; 1-3). The main consequences are: a) the belief that it is legitimate to base safety studies on analytical chemistry to determine the presence or absence of specific chemicals, and that data on their relative concentrations in e-liquids and emissions are sufficient to provide a quantitative measure of harm; b) the belief that the route of exposure has little effect on nicotine toxicity, and that, as few toxic effects have been observed since the times when various nicotine delivery devices were first introduced (ranging from 10 years for ECs to 30 years or more for nicotine replacement therapies (NRTs)), nicotine must be relatively inactive; and c) the belief that long-term toxicity can be predicted on the basis of acute effects.

The idea of deriving quantitative information on risk, while having only qualitative supporting data for ECs, originated from a Multiple-criteria decision analysis (MCDA) study (4). Our concerns about this are summarised in Table 2. Nutt et al. must have settled on 95% as a convenient comparative number, which PHE eventually simply expressed differently, ever since which the figure has been quoted ad nauseam, without any supporting data.

Overlooking the effect of route of administration is exemplified by a report of the Royal College of Physicians (4) which stated: There are, however, no grounds to suspect that inhaled nicotine will have an appreciably different risk profile from nicotine delivered via other routes of absorption. This statement is imprecise, and was not backed by any references. There are many reasons why toxicity can depend greatly on route, rather than merely on target organ(s). Another important factor is the possibility of drugs going into systemic circulation, once entering the body, usually by routes other than orally, before passing through the liver first - the organ which normally reduces systemic concentrations of parent compounds and which alters them to produce various metabolites, which can be more toxic or less toxic than the parent compound.

4. Superficial and inaccurate reporting of supporting evidence

A paper by Cheng (6), cited in a report commissioned by PHE, written by McNeill et al. (7), provides evidence of the presence in vapours of some potentially carcinogenic tobacco-specific nitrosamines (TSNAs) at widely different levels (RF), but McNeill et al. did not mention the evidence in relation to safety, even though they made some other statements on the issue. This contrasts with another PHE-Commissioned report focusing on safety, (8), authored by Britton and Bogdanovica. These authors did not mention the extensive analytical data for such chemicals, as Cheng’s paper was omitted in favour of one by Goniewicz et al describing that only very low levels of these chemicals are associated with ECS (9).

In a highly critical editorial (10), The Lancet noted that the PHE report was evidence-based confusion rather than being a “landmark review”, as referred to by Kevin Fenton, PHE’s Director of Health and Wellbeing. When commenting on a paper purporting to demonstrate a link between DNA damage in lung cells and exposure to EC vapour,
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Fenton, replied that Public Health England has always been clear that e-cigarettes are not 100 per cent safe and we will carefully consider this new study and continue to be vigilant. But our major world leading review, published recently, found that e-cigarettes carry a fraction of the risk of smoking’ [underlining added]….. (http://www.telegraph.co.uk/science/2016/03/12/e-cigarettes-are-no-safer-than-smoking-tobacco-scientists-warn/). The so-called ‘world-leading review’ by PHE was nothing of the sort – it was essentially a very poor appraisal of the situation.

We also note that, while Nutt et al., in 2014 (4), urged caution when interpreting their MCDA data, they supported PHE’s 95% safer value in a letter published two years later (11). The MCDA paper (4) is also superficial, especially with respect to criteria for calculating maximum relative harm (MRH) and on how the inescapable problem of the huge bias in data for tobacco smoking compared with ECs was corrected for. This bias is due to the much shorter time for which ECs have been available for use and for testing, meaning that more subjectivity would have been required when assessing ECs to reach consensus at the decision conference, an even greater problem in 2013, when the discussions took place. This problem was also noted in a review on ECs, published in April 2014 (12), which concluded that "Existing evidence suggests that these products [ECs] are by far a less harmful alternative ....", although it was admitted that only a very few toxicological studies were available.

Despite searching background literature on the MCDA technique, some of it recommended by Nutt et al., and after watching seminars (13-15) by the two leading authors, we have not found any convincing explanations for our concerns about MCDA. Other critics of the MCDA approach include: Kujawski (16), who commented that the specific MCDA model used can greatly influence the rankings of the alternatives for a given set of criteria; and Rolles and Measham (17), who were highly critical of the criteria and weighting used for ranking.

5. Nicotine - an inconvenient truth?

There is widespread agreement in the various reports supporting ECs that, apart from its addictivity, nicotine, is otherwise non-toxic at its in-use concentrations. Nicotine is actually one of the most toxicologically and pharmacologically active substances known (see reviews cited in ref 1). Structural alerts for DNA and protein binding were identified (unpublished studies by us, by using Toxtree, a decision-tree expert system for structure-activity relationships [SAR]), explaining the observed genotoxicity in the literature, and raising questions about respiratory sensitization (mediated by DNA binding), and other mechanically-related diseases, such as Chronic Obstructive Pulmonary Disease (COPD). Of interest is the fact that propylene glycol and glycerine lacked these alerts, although they might be precursors for toxic carbonyl compounds, the generated amounts of which increased with heater settings in one study (18), but it is possible to generate them without the excessive levels causing dry puff.

The literature on nicotine carcinogenicity and reproductive toxicity, reviewed by us in ref 1 [18 references cited therein], at the very least, suggests that, if not a complete carcinogen (acting as an initiator and promoter, nicotine acts on a variety of key post-initiation stages of the multi-step process of carcinogenesis (Fig. 1), including inhibition of apoptosis and immune system suppression, tumour promotion, cell proliferation, progression, stimulation of specific cell activating factors, angiogenesis, and the induction of unique patterns of
differential gene expression (see also 19). The drug also activates at least five mitogenic signaling pathways and cooperates with TSNAS toward the carcinogenic activity of tobacco smoke (20), and is also embryotoxic and modulates fertilization.

6. **Cardiovascular disease (CVD) effects**

Nicotine and ultra-fine fibres in the particulate matter in tobacco smoke have been implicated separately to be involved in smoking related CVD via their ability to induce inflammation in the endothelial layer in blood vessel walls, a first step in atherogenesis leading to CVD (21, 22). The fibres increase the surface area for reactive oxygen species (ROS), and possibly act also by causing some physical damage to the cells.

It is possible that the two components have to interact synergistically for an effect. Such a model would explain the lack of association between NRT usage and CVD, and would suggest that EC use would also not be linked to NRT, unless some other component could mimic the effect of the fibres. Candidates for this role are the nanoparticles generated from the heating elements in e-liquid reservoirs. Some of the fibres have overlapping dimensions with NPs (23), but their surface chemistry needs to be characterised, and further work is needed to see if they interact with nicotine to induce atherosclerosis. Interestingly, Zhao et al. (24) recently demonstrated ROS generation by e-cigarettes, which was highly dependent on brand, flavour, puffing pattern, and voltage.

7. **Basing the safety of nicotine on human studies of NRT users and snus takers**

Often, the results from epidemiological studies of users of NRT, and of smokeless tobacco (e.g. ‘snus’, which is popular in Scandinavia, the device being a pouch of tobacco, maintained in the mouth for extended periods), without increases in the incidence of conditions like cancer, COPD or CVD, in device-users compared with matched non-users, are used to argue against nicotine being toxic. However, such arguments fail to explain all of the evidence and/or do not accord with all of the facts.

While the 30-year or so period during which NRT products have been available would seem to be a sufficiently long time for the lack of increased susceptibility to cancer to be attributed to the non-carcinogenicity of nicotine, it is a collective figure for all users, which should not be confused with individual treatment durations for a course of NRT (typically 8-12 weeks per patient) – too short a duration for assuming non-carcinogenicity.

With regard to snus, careful reading of the statistics in the annual Swedish Cancer Registry (http://www.socialstyrelsen.se/english) reveals a complex relationship between snus-taking, lung cancer and other cancers. Two key conclusions from the statistics, a) that the use of snus almost halved lung cancer incidence in males in Sweden, and b) that it is not associated with increases in the occurrence of a range of other ‘common’ cancers, do not agree with all the available evidence, some of which suggests that snus usage has had only a minimal effect on lung cancer incidence overall, in males, and that increases in a range of other cancers (including oral and pancreatic) can be linked to exposure to snus.

Therefore, the statistics on the change in cancer incidence in relation to snus-taking in Sweden need to be interpreted carefully. Some other published analyses of population studies, including that by Lee et al. (25), essentially giving snus the all-clear, were criticised by Tomar et al. (26). Finally, if nicotine were a tumour promoter, a long period...
between exposure to an initiator and promoter is just what would be expected to still potentially result in tumorigenesis.

8. **An attempt to obtain long-term data on ECS**

A so-called ‘long-term’ biomonitoring study, published in March 2017 (27), allegedly demonstrating the much greater safety of vaping compared with smoking, has been hailed as being the closest yet to endorsing the 95% less harmful value and PHE’s policy on ECs.

Biomonitoring assesses internal exposure to, and the possible systemic effects of, a substance to which an individual is exposed, thereby strengthening the link between exposure and effect. The study in question analysed urine samples obtained from smokers, vapers and those on various types of other NRT devices, for the presence of biomarkers of exposure to several carcinogens found in tobacco smoke and linked to lung cancer. The key criterion for inclusion in the study was the daily use of the same broad category of device for at least six months prior to sampling. This allowed conventional NRT users to use devices with varying routes of administration, introducing a further source of variability. Levels of biomarkers were detected and quantified by using highly sensitive methods for chemical analysis.

The lowest concentrations of all the biomarkers were found in the samples from the EC-only users. As the differences were quoted as being between 90-100%, the authors interpreted this as vindication of the 95% figure.

However, the study was flawed in its rationale (it relied on chemical analysis), and its design (small numbers of volunteers and wide differences in gender ratios between some of the cohorts and only one timepoint). Conventional long-term toxicity testing involves repeat exposure studies and continual surveillance of laboratory animals, for at least several months. The tests are designed to detect chemicals that might not specifically exhibit acute effects. Therefore, this study, with only one sampling, should not be regarded as being equivalent to a repeat-dose toxicity study. There was also no control of fluid and nutrient intake on the day of sampling, let alone of the type of device, and no determination of the various e-liquid compositions. At best, the study could have provided only a snapshot of what was happening during the period involved.

9. **A role for non-animal methods**

Regulatory test batteries for new drugs include subchronic and chronic tests that are specifically designed to predict repeat-dose toxicity (<90 days) and longer-term toxicity, some studies of which take some 2-3 years to complete. Long-term models of respiratory diseases also exist (2, 28). An example of one of these has recently been published (29), in which mice were exposed by inhalation to nicotine-containing EC fluids for one hour daily over four months. The exposures induced effects associated with the onset of COPD, including cytokine expression, airway hyper-reactivity, and lung tissue destruction. These effects were nicotine-dependent in the mouse lung, suggesting that inhaled nicotine contributes to airway and lung disease.

However, our suggestion of the need for more hazard data for ECs does not necessarily mean more animal testing, since many *in vitro* methods exist (see citations in refs 1-3)
These offer many advantages over their in vivo counterparts, ranging from more-precise dosimetry to advantages in data interpretation. This is especially true for inhalation testing (28).

Monolayer-cultures of cells from target airway sites can be used. For example, in the four-month COPD study mentioned above, the same results were obtained when normal human bronchial epithelial [NHBE] airway cells were cultured at an air-liquid interface (ALI) and exposed to EC vapours or nicotine solutions by using a Vitrocell smoke exposure robot.

It should be possible to obtain more-reliable and more-relevant data expeditiously through the application of integrated testing strategies involving advanced human cell-based tissue culture systems, in which their differentiated status is retained in culture, and which are representative of the major target sites in the airways for respiratory toxicity and disease, by using ALI exposure. Moreover, some of the toxicity endpoints (e.g. DNA damage) can be measured in situ in the tissue construct (several reviews have been published over the past year).

The tobacco industry has been active in this area, holding workshops and various integrated tiered testing strategies have emerged for improving and expediting hazard identification. We present a generalized strategy, based on this type of approach (Figure 2). The strategy also includes a repeat-dose toxicity testing stage involving the use of hollow fibre technology for maintaining the longevity of cells in culture by replacing spent culture medium with fresh medium.

It is also possible to develop in vitro micro-culture models of whole organs, in order to predict the effects of exposure at several different sites within the same organ, simultaneously. A pertinent example is a small 'airway-on-a-chip' device developed by Benam and coworkers (30). This system is lined by living human bronchiolar epithelium from normal or COPD patients. The device is connected to an instrument that delivers whole cigarette smoke in and out of the chips, to permit the study of smoke-induced pathophysiology in vitro.

10. Smoking cessation versus nicotine quitting

We also note that the rationale for NRT was originally geared toward the ultimate goal of detoxication from nicotine drug dependency. In other words, it was intended that treatment would progress from a phased withdrawal, from dual usage via exclusive NRT usage to no usage. The current emphasis ion smoking cessation is regrettable, since it would greatly prolong exposure to nicotine. While this might not increase drug dependency, it could result in many other adverse effects, including tumour promotion and progression of initiated cells already formed in smokers before they started to quit.
11. Discussion

The argument for encouraging the use of ECs is based on: a) the apparent lack of association between nicotine exposure and carcinogenesis, CVD and other respiratory diseases, interpreted as meaning that they can be regulated lightly by waiving the batteries of preclinical and clinical tests to which most new medicines are subjected; b) an estimate with no scientific basis that vaping is 95% less harmful than tobacco smoking; and c) the belief that the focus should be on achieving tobacco smoking cessation, rather than drug independence. Our investigations have encouraged us to conclude that all these assumptions are spurious when considered with respect to principles of toxicology involving hazard prediction and risk assessment.

The safety assessment of ECs should, in principle, be no different from that required for other new medicines. No good reasons for by-passing the risk assessment and risk-benefit procedures normally required for registering pharmaceuticals have been made public, and we also note that PHE mandated itself to publish its decision, without first having a public consultation stage.

We also consider that the use of panels of experts to decide, largely on the basis of opinion and value judgment, especially for ECS, about the 'relative harms' of nicotine-release devices, without relevant and reliable quantitative data about the harms resulting from exposure especially to EC vapour, was unwise and unnecessary, especially when non-animal testing strategies are available to generate meaningful hazard information and to fill data gaps, to be used, with other information, in a convincing weight-of-evidence assessment.

Finally, we stand by our belief, expressed in a letter published in The Times on 18 February 2016, that “The human respiratory system is a delicate vehicle, on which the length and quality of our lives depend. For governments and companies to condone, or even suggest, the regular and repeated inhaling of a complex mixture of chemicals with addictive and toxic properties, but without comprehensive data, is irresponsible and could have serious consequences.”

12. Recommendations

1. Good Manufacturing Practice guidelines should specify device design, capability, construction, mode of nicotine delivery and permissible ingredients, and their maximum amounts.
2. The designs should avoid the potential for excessive customisation.
3. Professional toxicologists should be involved in advising on safety issues relating to regulation of the use of ECs.
4. The intrinsic risks from vaping should be investigated and calculated separately, before comparison with the risks from tobacco smoking.
5. ECs should be considered as NRT products, rather than for prolonged recreational usage, until more long-term safety data have become available.
6. The toxicity of nicotine should be investigated further, as should the ability of nanoparticles in EC emissions to mimic the effects on CVD of particulate matter in tobacco smoke.
7. Threshold values for nicotine toxicity should be identified.
8. The end-game should be total cessation of the use of nicotine, beginning with tobacco smoking, but proceeding to cessation of the use of NRTs and ECs.

9. POS (point of sale) literature should emphasise the importance of nicotine quitting.

10. The MHRA should be more transparent about how ECs will be regulated via a 'light-touch" approach, especially by applican of the concept of bioequivalence.

11. We strongly urge that further in vitro methods for detecting long-term toxicity and chronic disease conditions as a result of inhalation, should be developed and validated and accepted for use as soon as possible.

12. Several prospective long-term epidemiological studies should be initiated in the near future, to assess the adverse clinical and toxic effects from vaping. These should involve biomarkers of exposure and effect, such as DNA adducts, chemically-modified bases, and genotoxicity of body fluids.

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13. References


14. Tables and figures:
Table 1
Ten principles of toxicology being ignored in the debate in the UK concerning electronic cigarettes

1. There can be synergistic or antagonistic effects between constituents of complex mixtures.
2. Non-linear dose-responses are often exhibited.
3. There can be lag periods of many years between exposure and effect, e.g. for some cancers.
4. Analytical chemistry is of limited value for predicting non-toxicity.
5. Some chemicals and endpoints lack thresholds of toxicological concern, and toxicity can occur at very low concentrations.
6. Long-term effects are just as important as acute ones.
7. Quantitative expressions of safety should *always* be based on numerical data.
8. Route of entry/administration can have a large effect on toxicity.
9. Acute toxicity data should be used with great care, when attempting to predict long-term effects.
10. When in doubt, adopt the precautionary approach.
### Table 2

Ten problems with the MCDA study *(Nutt et al., 2014)* on Maximum Relative Harms (MRHs) for nicotine devices, and its subsequent interpretation

<table>
<thead>
<tr>
<th>Problem</th>
<th>Description</th>
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<tbody>
<tr>
<td>1.</td>
<td>Insufficient information available to repeat study closely with a completely different panel of experts (e.g. criteria for MRH unclear).</td>
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<tr>
<td>2.</td>
<td>Panel did not have on it a toxicologist experienced in risk assessment (the focus was not on comparison of hazard compared with exposure).</td>
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<tr>
<td>3.</td>
<td>Huge bias of harm information available for tobacco smoking compared with vaping, (meaning much more conjecture in scoring the latter).</td>
</tr>
<tr>
<td>4.</td>
<td>Therefore, although scoring in general based on opinion, this would have been less so for tobacco smoking.</td>
</tr>
<tr>
<td>5.</td>
<td>Since 2013/4, much more safety data have become available for vaping, and such information should inform fresh new discussions.</td>
</tr>
<tr>
<td>6.</td>
<td>No explanation as to how consensus was achieved between the panelists (no proceedings of face-face workshop).</td>
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<tr>
<td>7.</td>
<td>No numerical hazard data to support the quantitative estimate made for relative harm of vaping versus tobacco smoking (resulting in a false impression of accuracy).</td>
</tr>
<tr>
<td>8.</td>
<td>Insufficient focus on the toxicity of nicotine and its contribution to harms (leading to a possible under-estimation of harm from vaping).</td>
</tr>
<tr>
<td>9.</td>
<td>Harms from smoking based on short-term and chronic effects, whereas, for vaping, no chronic data available (long-term safety cannot be accurately predicted from acute effects).</td>
</tr>
<tr>
<td>10.</td>
<td>MRH values were based on wide range of criteria, other than safety <em>per se</em>,** meaning use of the term ‘harm’ in the paper is misleading (‘harm’ has been used to infer safety, when the terms are not synonymous).</td>
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</tbody>
</table>

* reference 4 in References

**only 5/14 harm criteria were related to personal user adverse effects, and one of these was drug dependency;
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Figure: 1 Multi-stage carcinogenesis – effects of tobacco smoke (TS) & nicotine (NIC)

- **Initiation**: Normal cell
  - Spontaneous or induced genotoxicity; DNA damage; mutation; epigenetic changes; oncogene activation; change in cell surface antigens; accumulation of genetic changes
  - TS - complete carcinogens
  - Nic - genotoxic; induces DNA damage, subject to repair, but Ames negative

- **Promotion**:
  - Non-genotoxic processes; receptor binding; growth factor synthesis; stimulation of signalling cellular cascades; cell division
  - Nic binding to nicotinic acetylcholine receptors induces many processes caused by tumour promoters & immunosuppression
  - Normally loss of initiated cells by immune attack, & apoptosis but NIC blocks this

- **Progression**: Cell promotion
  - Apoptosis; de-differentiation; clonal proliferation; new gene expression; growth factor stimulation and >5 signalling pathways stimulated. angiogenesis
  - Loss of promoted cells by immune attack, & apoptosis

- **Malignancy**: Primary tumour
  - New gene expression: Cell detachment; migration; metastasis and malignancy
  - Secondary tumour
  - Teratogenic in vitro; embryotoxic; gametogenesis slowed; Fertilisation inhibited; miscarriages down
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1. TEST MATERIAL
   e.g. new aerosol/e-liquid/vapour/nanoparticle/additive

2. CHEMICAL ANALYSIS
   prioritisation only

3. (QUANTITATIVE) STRUCTURE-ACTIVITY RELATIONSHIPS ((Q)SAR)
   used with algorithms for potential constituent interactions

4. TOXICITY TO HUMAN MONOLAYER
   CELL CULTURES OF TARGET TISSUE

5. TARGETED GENE EXPRESSION PROFILING

6a. 3-D ORGANOTYPIC HUMAN CELL-BASED TOXICITY TO
   TISSUE CONSTRUCTS OF ALL KEY SITES
   OF RESPIRATORY SYSTEM
   growing at ALI and in situ effects like DNA damage, micromuclei, inflammatory

6b. REPEAT-DOSE STUDIES
   e.g. hollow fibre systems

7. HUMAN/CLINICAL STUDIES IN VOLUNTEERS
   using microdosing and biomarkers of internal exposure
   (e.g. primary metabolite) and effect (e.g. genotoxic metabolites)
   in urine/blood, etc.

8. MHRA
   regulatory decision
   weight-of-evidence approach and termination of testing after stages 3, 4 and 6

FIGURE 2 A GENERALISED INTEGRATED TOXICITY TESTING STRATEGY FOR E-CIGARETTES