

Impact case study (REF3b)

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| Institution: Swansea University |
| Unit of Assessment: 3a - Allied Health Professions, Dentistry, Nursing and Pharmacy |
| Title of case study: The concept of genotoxicity thresholds benefits patients and the pharmaceutical industry |
| 1. Summary of the impact |

Genotoxicity (DNA damage) can often induce carcinogenesis. Swansea-led work on 'genotoxicity thresholds' reassured 25,000 HIV-infected individuals, who had taken anti-viral tablets (Viracept®) contaminated with the genotoxin ethyl methanesulfonate (EMS).

Before 2008, genotoxicity was assumed to increase with dose, and genotoxic drugs were discarded. Research at Swansea University showed that exposure to low-levels of genotoxins did not pose significant risks to DNA. This concept has now been incorporated into regulatory guidelines; in July 2008 the European Medicines Agency accepted that cancer-risk was not increased for patients who received Viracept® tablets contaminated with a low dose of the genotoxin ethyl methanesulfonate (EMS).

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| 2. Underpinning research |
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The regulatory safety assessment of drugs requires investigation of their potential to induce DNA damage (genotoxicity). This is important as DNA damage drives cancer development. The genotoxicity of drugs is determined using *in vitro* and *in vivo* tests, and from the 1970s until 2008, DNA damage had been assumed to be induced in a linear manner by genotoxic compounds. So, a drug was deemed to be either genotoxic or not. Drugs with any degree of genotoxicity were usually discarded, because of concerns about potential cancer risk, in favour of non-genotoxic ones, even when the latter were less efficacious. This has commonly led to the discarding of valuable drugs at late stages of drug development ('late stage attrition').

The concept of genotoxicity thresholds was introduced by the late Professor James Parry and colleagues at Swansea University in the mid 1990s [R1, R2]. Swansea University led theoretical discussions about whether thresholded dose responses existed for genotoxic agents [R3]. The threshold model would mean that "safe" low doses could exist. This sparked major controversy in the field, with some arguing that linear models should remain the standard, if only as a precaution. Much of the "linear" argument stemmed from estimations of the risk of genotoxicity associated with radiation, which have not always been relevant to chemical genotoxicity and have themselves been questioned (Calabrese *et al.* "Key studies used to support cancer risk assessment questioned", *Environmental and Molecular Mutagenesis* 2011. [doi:10.1002/em.20662](https://doi.org/10.1002/em.20662)). Crucially, until 2007, there were few data to inform the argument. Gareth Jenkins joined Parry's group in 1994 and with Parry (retired in 2005) and colleagues at Swansea has kept the concept of genotoxicity thresholds on the research agenda [R3] and provided the first data to prove that genotoxicity thresholds exist for drugs [R4].

A programme of research on the dose responses to known genotoxins *in vitro* was initiated in 2000. Professor Parry (Professor of Genetics until 2005) and Gareth Jenkins (Senior Lecturer from 2004, Professor from 2010) led the work, Shareen Doak (Post-doctoral researcher 2004-2006, Lecturer 2006-2010, Reader from 2012) and George Johnson (PhD student 2002-2006, tutor 2006-2008, Lecturer 2008-2012, Senior Lecturer from 2012) carried out the initial work. The work focused on genotoxic dose response curves for 4 alkylating agents that are classic genotoxic chemicals. Work carried out between 2001 and 2006 demonstrated for the first time that 2 of the 4 alkylating agents (EMS and MMS) had thresholds for both chromosome and DNA base mutation *in vitro* [R4]. These data resulted in a paradigm shift in July 2008: the acceptance of genotoxicity thresholds and the concept of "safe" exposure levels to some genotoxic drugs by the scientific community as a whole, and changes to international guidelines from major regulatory agencies (see below). This work has had a broad international reach.

The study of genotoxicity thresholds remains a research focus at Swansea University [R5, R6]. Our lead role in validating thresholds for genotoxins has led to more than 15 peer-reviewed publications, and £1.8 million in research grant income from Research Councils and industry (see Section 3).

3. References to the research

Key references Swansea researchers are highlighted in bold. Information on journals' impact factor (IF) and ranking among the 83 Toxicology journals (unless stated otherwise) in 2012 is provided for all references. Citation counts by August 2013 according to Web of Knowledge are provided.

- R1. **Parry JM**, Fielder RJ, McDonald A (1994). Guidelines for testing of chemicals: Thresholds for aneuploidy-inducing chemicals. *Mutagenesis* **9**:503–504. [DOI: [10.1093/mutage/9.6.503](https://doi.org/10.1093/mutage/9.6.503)] IF 3.2, Journal ranked 21st. 31 citations.
- R2. **Parry JM, Jenkins GJS, Haddad F, Bourner R, Parry EM.** (2000). In vitro and in vivo extrapolations of genotoxin exposures: consideration of factors which influence dose-response thresholds. *Mutat. Res.* **464**:53–63. [DOI: [10.1016/S1383-5718\(99\)00166-7](https://doi.org/10.1016/S1383-5718(99)00166-7)] IF: 3.0, Journal ranked 23rd. 28 citations.
- R3. **Jenkins GJS, Doak SH, Johnson GE, Quick E, Waters EM, Parry JM** (2005). Do dose response thresholds exist for genotoxic alkylating agents? *Mutagenesis* **20**:389–398. [DOI: [10.1093/mutage/20.4.389](https://doi.org/10.1093/mutage/20.4.389)] IF: 3.2, ranked 21st. 37 citations.
- R4. **Doak SH, Jenkins GJS, Johnson GE, Quick E, Parry EM, Parry JM** (2007). Mechanistic influences for mutation induction curves following exposure to DNA-reactive carcinogens. *Cancer Res.* **67**:3904–3911. [DOI: [10.1158/0008-5472.CAN-06-4061](https://doi.org/10.1158/0008-5472.CAN-06-4061)] IF: 7.8, ranked 12th among 194 Oncology journals. 84 citations.
- R5. **Johnson GE, Doak SH, Griffiths SM, Quick EL, Skibinski DO, Zoulikha M Zair ZM, Jenkins GJ** (2009). Non-linear dose–response of DNA-reactive genotoxins: Recommendations for data analysis. *Mutat. Res.* **678**:95–100. [DOI: [10.1016/j.mrgentox.2009.05.009](https://doi.org/10.1016/j.mrgentox.2009.05.009)] IF: 3.0, ranked 23rd. 20 citations.
- R6. **Zair ZM, Jenkins GJ, Doak SH**, Singh R, Brown K, **Johnson GE** (2011). N-methylpurine DNA glycosylase plays a pivotal role in the threshold response of ethyl methanesulfonate-induced chromosome damage. *Toxicol. Sci.* **119**:346–358. [DOI: [10.1093/toxsci/kfq341](https://doi.org/10.1093/toxsci/kfq341)] IF: 4.65, ranked 9th. 3 citations.

Grant income stemming from research on genotoxicity thresholds

1. Unilever PLC project grant (PI: G Jenkins). Non-linear DNA damage dose responses. 2008–2012. £295,987.
2. Roche project grant (PI: G Johnson). Mechanistic investigations into the non-linear genotoxic dose response of ethyl methanesulphonate (EMS), in the low dose region of exposure. 2008–2010. £188,538.
3. Medical Research Council (MRC) grant (PI: S Doak). Understanding the genotoxic potential of ultra-fine superparamagnetic iron oxide nanoparticles. 2008-2011. £473,964.
4. MRC CASE studentship with Astra Zeneca (PI: G Jenkins). To establish genotoxic thresholds for selected DNA-reactive Agents. 2010–2013. £73,370.
5. National centre for the replacement, refinement and reduction of animals in research (NC3Rs) studentship (PI: G Jenkins). The validation of defined genotoxic thresholds, leading to better in vitro risk assessments of carcinogenic potential. 2010–2014. £120,000.
6. MRC fellowship to Gareth Jenkins (PI: G Jenkins). Development of 3D human tissue models for toxicological testing. 2010–2012. £65,793.
7. Engineering and physical sciences research council (EPSRC) CASE studentship with GlaxoSmithKline (GSK) (PI: G Johnson). Development of the high throughput Pig-A mutation assay *in vitro*. 2011–2015. £73,370
8. NC3Rs project grant (PI: G Jenkins). In vitro predictions of human carcinogenicity. 2012–2015. £384,179.

4. Details of the impact

Underpinning research at Swansea provided comprehensive evidence that some genotoxic drugs have thresholds for genotoxicity. Two levels of international impact resulted from this research: (1) peace of mind offered to patients involved in the Viracept® case worldwide; (2) international regulatory changes relating to low-level genotoxin contamination.

Reassuring patients who took contaminated Viracept®^{C1}. Work at Swansea has had an impact on 25,000 HIV-infected individuals in 29 countries who had received an anti-viral medication

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(Viracept®) containing the genotoxic agent EMS [C2]. In 2007, Roche reported that a batch of Viracept® had been contaminated with EMS during manufacture and withdrew the drug from the market [C1, C3]. This incited fears among HIV-infected individuals on antiretroviral therapies:

“The recent recall of [Roche's](#) antiretroviral drug Viracept® has ‘created panic’ among HIV-positive people taking antiretrovirals in Zambia, some of whom believe that other drugs might not be safe.” [C4]

Researchers at Swansea University had previously shown that the genotoxicity of EMS had a threshold dose. Following detailed discussions with Jenkins and colleagues, Roche launched an *in vivo* study to investigate the genotoxic dose responses of EMS in a mouse model. According to one of their main publications on the Viracept® incident:

“a research group from the University of Swansea, UK, had published an important article in this context, which appeared in Cancer Research on 15 April 2007. This article yielded reliable evidence for a threshold for chromosome damage and mutations induced by EMS in a human lymphoblastoid cell line in vitro. These data encouraged Roche to approach risk assessment for Viracept® patients.” [C9]

The Roche study confirmed that there was a threshold for the genotoxicity of EMS *in vivo*. The newly defined threshold dose in mice allowed extrapolation to a safe human exposure level. This revealed that the HIV-infected individuals taking the contaminated tablets were not at increased risk of mutagenesis (and hence cancer). In July 2008, EMA accepted these data as proof that patients were not at an elevated risk of cancer [C2]. Hence, the impact of the work was first felt in 2008. Patients and professionals were informed of the decision and therefore reassured in 2008.

Swansea's threshold work over the preceding 8 years was central to this final decision. Without that original research on genotoxicity thresholds *in vitro* this approach would not have been envisaged by Roche: *“The original work in vitro published by Shareen Doak and colleagues in Cancer Research in 2007 was the ultimate and indispensable trigger for Hoffmann-La Roche to enter further work in vivo to conduct a proper risk assessment for HIV patients who have ingested Viracept® tablets contaminated for some period in 2007 with EMS. Based on the studies in vitro conducted in Swansea under supervision of the late Prof Jim Parry, Roche was successful in proving a threshold in vivo This reasoning was accepted by many authorities around the world after having been presented to the EU authorities in 2008.”* Lead, Full Development Projects for Toxicology, Hoffman-LaRoche Ltd. [R4, C11]. This risk assessment allayed the fears of the 25,000 individuals globally who had taken the contaminated tablets and allowed their continued treatment with these valuable anti-viral drugs [C3].

This also saved Roche from financial compensation claims and the cost of setting up a patient registry to monitor these 25,000 patients, two activities estimated to cost more than \$100 million.

Informing international regulatory policies

The impact of the acceptance of genotoxicity thresholds continues to be felt in the updates to regulations on safety assessments of drugs and chemicals. For example, the UK Government Committee on Mutagenicity (COM)'s statement on thresholds for *in vivo* mutagens [C5] and other COM reports have cited threshold work from Swansea University [C6-7], and the European Food Standards Agency (EFSA) has issued a report on genotoxicity testing strategies [C8] that also notes the issue of thresholds and cites the pioneering research undertaken at Swansea University. Both these advisory/regulatory bodies cite Swansea-led work as evidence for the existence of thresholds and propose accepting licensing of compounds with thresholded genotoxicity.

Key members of the Swansea research team have also been invited to join or lead policy shaping mutagenicity committees [C10]. In 2009 Gareth Jenkins was invited to join the UK Government's Committee on Mutagenicity (COM) [C10]; in 2013 Shareen Doak was invited to join COM [C10]. In 2012 George Johnson chaired the Genetic Toxicology Technical Committee (GTTC) Quantitative Work Group of the International Life Sciences Institute (ILSI). These activities have helped to disseminate our key findings and to educate policy-makers about the importance and relevance of thresholds for genotoxicity.

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5. Sources to corroborate the impact

- C1. General overview of Viracept® case. <http://www.aidsrestherapy.com/content/6/1/18> (2009).
- C2. EM(E)A document on conclusions of Viracept® incident & numbers of patients affected: http://www.emea.europa.eu/docs/en_GB/document_library/Medicine_QA/2009/11/WC500015048.pdf (July 2008).
- C3. Report by committee for medicinal products for human use (CHMP) citing Swansea work on p11, section 2.2.1. paragraphs 1 and 3: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/000164/WC500050681.pdf (September 2007).
- C4. IRIN news reports: <http://www.irinnews.org/report/72816/zambia-aids-drug-recall-creates-panic> (2007).
- C5. UK Government's Committee on Mutagenicity (COM) statement on thresholds citing role of work at Swansea on p1 (1) & (4), p2 (7) & (9), p3 references 1 & 3: <http://www.iacom.org.uk/papers/documents/COMThresholdsinmutagenicityMUT0902.pdf> (2010).
- C6. Commentaries from 2001 COM meeting on genotoxicity thresholds citing role of Swansea: <http://www.iacom.org.uk/statements/COM01S3.htm> (June 2001).
- C7. Two COM documents relating to papers and presentations by Gareth Jenkins (Swansea): http://www.iacom.org.uk/papers/documents/mut0617_000.pdf (2006). <http://www.iacom.org.uk/papers/documents/Thresholdsforin-vivomutagensMUT09.08.pdf> (Sept 2008).
- C8. European Food Standards Agency (EFSA) report on genotoxicity and the existence of thresholds, citing the role of Swansea University in the development of the genotoxicity threshold concept and genotoxicity testing strategies applicable to food and feed safety assessment. Section 8.1 of document cites Parry *et al.* (1994), Doak *et al.* (2007) and Jenkins *et al.* (2010): <http://www.efsa.europa.eu/en/efsajournal/pub/2379.htm> (September 2011).
- C9. Roche publications on Viracept® and EMS, citing Doak *et al.* (2007):
Mueller L, Singer T (2009). EMS in Viracept — The course of events in 2007 and 2008 from the non-clinical safety point of view. *Toxicol. Lett.* **190**: 243–247. [DOI: [10.1016/j.toxlet.2009.02.005](https://doi.org/10.1016/j.toxlet.2009.02.005)]
Gocke E, Ballantyne M, Whitwell J, Mueller L (2009). MNT and MutaMouse studies to define the in vivo dose response relations of the genotoxicity of EMS and ENU. *Toxicol. Lett.* **190**: 286–297. [DOI: [10.1016/j.toxlet.2009.03.021](https://doi.org/10.1016/j.toxlet.2009.03.021)]
Gocke E, Mueller L (2009). In vivo studies in the mouse to define a threshold for the genotoxicity of EMS and ENU. *Mutat. Res.* **678**:101–107. [DOI: [10.1016/j.mrgentox.2009.04.005](https://doi.org/10.1016/j.mrgentox.2009.04.005)]
- C10. Chair, Committee on Mutagenicity (COM). Can confirm role of Swansea group in Threshold paradigm shift and membership of the committee.
- C11. Lead, Full Development Projects for Toxicology, Hoffmann-La Roche Ltd. Can confirm role of Swansea group in threshold paradigm shift and that being the basis for the Viracept research at Roche that allayed the fears of affected patients.