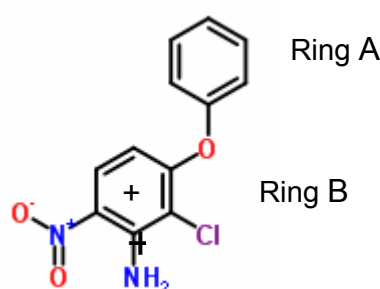


COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COM)**STATEMENT ON THE REVIEW OF MUTAGENICITY OF ACLONIFEN AND RISK ASSESSMENT OF ITS POSTULATED METABOLITES (HYDROQUINONE AND PHENOL)****INTRODUCTION**

1. The COM has been asked for advice by the Pesticides Safety Directorate (PSD) on a pesticide active ingredient new to the U.K. which is undergoing evaluation through the independent Advisory Committee on Pesticides (ACP). The referral statement was as follows: 'ACP requested advice on the mutagenicity of Aclonifen and the genotoxicity risk assessment of the postulated metabolites hydroquinone and phenol. The referral does not include carcinogenicity data or the evaluation of mode of action for tumours in rodents observed in long-term carcinogenicity bioassays with Aclonifen'.
2. Aclonifen (2-chloro-6-nitro-3-phenoxyaniline) (figure 1.) is a selective systemic herbicide used for pre-emergence control of grass and broad leaved weeds in a range of crops.



+ = position of uniformly radiolabelled phenoxyaniline ring

Figure 1. Aclonifen

3. On 31 January 2008, at the request of the ACP Chair, an approach was made by PSD to the COM Chair for advice as to whether DNA adducts could be detected and measured in the existing stored tissues from animals dosed with Aclonifen. The COM Chair advised that it would not be advisable to undertake a retrospective analysis of stored tissues from Aclonifen treated animals for DNA adducts.
4. A teleconference was held between the data holder (Bayer CropScience), PSD and Health Protection Agency (HPA) (representing COM Secretariat) on the 13 June 2008. The comments raised by HPA during this teleconference outlined the particular need to address the metabolism of Aclonifen to hydroquinone and phenol and the assumption that these two metabolites were non-threshold *in-vivo* mutagens.^{1,2} Subsequent to the

teleconference, the data holder submitted a revised position paper on 13 August 2008 on the relevance of phenol and hydroquinone formation following Aclonifen exposure which outlined their evaluation of the genotoxicity data on Aclonifen and metabolism of Aclonifen. ACP requested advice from COM on 30 July 2008.

5. The COM Secretariat held a teleconference with the data holder on 11 September 2008 to explain COM procedures, the referral for advice from ACP, data that COM would consider and to outline the procedures during committee with regard to a presentation from the data holder. Information on the possible areas of Aclonifen evaluation which COM Members might wish to raise questions was outlined, although it was noted that other aspects of Aclonifen might be raised.

6. The data holder submitted a presentation for the COM meeting on 13 October 2008 which was circulated to Members. In addition, on 21 October 2008 the data holder submitted a revision to the report dated 16 July 2004 on cleavage of the diphenyl ether bond in the Aclonifen molecule which had been circulated to Members.³ The revised report was circulated to COM Members and replaced the aforementioned 2004 report.

7. The data holder attended the COM meeting of 23 October 2008 to make a short presentation and answer COM queries regarding the evaluation of the metabolism and mutagenicity of Aclonifen.

COM CONSIDERATION OF AREAS FOR DISCUSSION

8. The COM considered the submitted data, which included an extract from the detailed record of ACP consideration of Aclonifen at ACP meeting 329, extracts from draft EU assessment report on metabolism and genotoxicity of Aclonifen, which presented information on structure, use as a pesticide, ADME studies, toxicology, mutagenicity, carcinogenicity and reproduction, data from mutagenicity test reports on Aclonifen, copy of the report on the investigation of the potential for DNA-binding of Aclonifen and the revised position paper from the data holder on the cleavage of the diphenyl ether bond of Aclonifen.³⁻¹³

9. The Chair asked COM to consider the questions to ask the data holder and proposed Members should first consider the metabolism of Aclonifen followed by the mutagenicity data on Aclonifen. The discussion of mutagenicity data focussed on determining whether it was possible that the potential genotoxic effects of hydroquinone and phenol formed from Aclonifen could be assessed in these studies.

The areas for discussion related to:

10. Evaluation of data for the metabolism of Aclonifen and evidence for the systemic formation of hydroquinone and phenol from absorbed Aclonifen. It was noted that there were published papers in the peer reviewed scientific

literature which provided examples of diphenyl ether breakage in a variety of species.¹⁴⁻¹⁷

11. Evaluation of the comparisons made by the data holder between mutagenicity data on hydroquinone and phenol with Aclonifen.
12. Evaluation of the mutagenicity testing strategy used by the data holder and specifically the reasons for undertaking an *in-vitro* rather than an *in-vivo* rat liver UDS study.
13. The COM noted the evaluation of carcinogenicity data was not included in the referral to COM, but agreed the data holder should be asked if there were data on tissue exposure from the carcinogenicity studies which might assist in evaluation of the mutagenicity data.

DATA HOLDER PRESENTATION

14. The data holder was asked to make a short presentation to the COM and to answer Members' queries.
15. The data holder presented an overview of Aclonifen rat metabolism studies, genotoxic potential of Aclonifen and their conclusions on the genotoxicity of Aclonifen.
16. Aclonifen had been ¹⁴C-labelled on the phenoxyaniline ring (B) but no radiolabelled studies had been undertaken with the phenyl ring (A) (figure 1.). The data holder noted that Aclonifen was rapidly absorbed via the oral route of administration and extensively metabolised with the majority of administered material (>90%) eliminated in the first 24 hrs via urine for both single dose and repeat dose studies (at 30 mg/kg bw). Approximately 40-48% of the absorbed dose was eliminated via the bile following an oral dose of 30 mg/kg bw. Tissue levels of radioactivity were very low. Aclonifen was metabolised by hydroxylation, methylation, reduction of the nitro group, N-acetylation, cleavage of the diphenyl ether bond and phase II conjugations. Potential diphenyl ether breakage had been inferred from the formation of glucuronide and sulphate metabolites from ring B. The data holder noted there were uncertainties in determining the total potential diphenyl ether bond breakage but overall this was estimated to be 9.2% in males and 7.3% in females. The data holder noted there was no evidence for cleavage metabolites in the repeat dose metabolism study and proposed that it was necessary for Aclonifen to be hydroxylated, and glucuronidated and sulphated before diphenyl ether breakage to form the conjugated forms of hydroquinone and phenol. This would provide an explanation for the negative findings in genotoxicity tests with Aclonifen.
17. With regard to the available mutagenicity studies on Aclonifen, negative results had been obtained in Ames tests, an *in-vitro* chromosome aberration study in human lymphocytes, an *in-vitro* gene mutation study in V79 cells (HPRT locus), and an *in-vitro* rat liver UDS assay. Negative results had also been obtained in a mouse micronucleus test using the oral route of

administration and no evidence for DNA binding in liver and urinary bladder had been reported in mice dosed orally with ¹⁴C-labelled Aclonifen (labelled in ring B). The data holder considered the higher concentrations used and evidence for reduced toxicity in the presence of exogenous metabolic activation in *in-vitro* mutagenicity studies in mammalian cells suggested that Aclonifen was being metabolised. The data holder noted that hydroquinone and phenol had given positive results in comparable studies for clastogenicity and gene mutation in V79 cells. In particular, Aclonifen was negative in an *in-vitro* rat liver UDS study where metabolism would have been expected. In addition, phenol and hydroquinone were positive in *in-vitro* UDS tests in Syrian Hamster Embryo (SHE) cells at dose levels almost 100-fold lower than tested with Aclonifen. The data holder noted the negative *in-vivo* oral mouse bone marrow micronucleus test (high dose level 7260 mg/kg bw) with Aclonifen and compared this with evidence for positive results in studies with hydroquinone (80 mg/kg bw) and phenol (265 mg/kg bw). The data holder concluded that Aclonifen was not genotoxic and that, if hydroquinone and phenol were formed during the metabolism of Aclonifen, then the results of the oral micronucleus test in mice should have been positive. The data holder drew the attention of COM to the detailed supporting slides in the presentation.

COM QUESTIONS FOR DATA HOLDER

18. A summary of the response given by the data holder on the areas for discussion is given below.

19. The data holder commented there were no specific data available on the formation of hydroquinone and phenol from Aclonifen. The data holder had considered the aspect of there being no evidence for diphenyl ether breakage of Aclonifen in the repeat dose study and suggested the breakage metabolites in the single dose studies being artefacts of mass spectrometry in these studies as one possibility for this observation. It was unlikely that there were individual animal data for the diphenyl ether breakage metabolism of Aclonifen as samples had been pooled prior to analysis and thus no assessment of the potential extent of inter-animal variation in metabolism could be made. With regard to the potential metabolism of Aclonifen to hydroquinone and phenol in exogenous metabolic fractions used in mutagenicity tests, the data holder considered the higher doses used and evidence for reduced toxicity in the presence of S-9 (compared to tests in the absence of S-9) in *in-vitro* mutagenicity studies in mammalian cells with Aclonifen provided some reassurance that exogenous metabolism had occurred although there were no specific data on metabolites formed. Members considered that alternatively it was possible that protein binding occurred in the presence of exogenous metabolising fractions reducing the dose available to cells.

20. The data holder commented that the comparisons of mutagenicity data on Aclonifen and that available on hydroquinone and phenol were based on the best available data and acknowledged that there were uncertainties, for example comparing different cell lines, and historic data from different

laboratories. The COM considered there were likely to be quite substantial differences in metabolic competency between SHE cells (used for tests with hydroquinone and phenol) and primary rat liver cells (used for the test with Aclonifen). In addition, differences in solubility of the test materials in vehicles used would also affect any comparison of the mutagenicity data. With regards to mutagenicity testing strategy, the data holder noted the rationale used for undertaking an *in-vitro* rather than an *in-vivo* rat liver UDS study was based on decisions on testing strategy reached at the time of testing rather than the specific question of *in-vivo* metabolism of Aclonifen to hydroquinone and phenol.

21. The data holder considered there were no relevant data from the carcinogenicity studies with Aclonifen on tissue concentrations in carcinogen target tissues (brain female rat), urinary bladder (mouse)) which might assist in the understanding of potential genotoxicity of Aclonifen.

22. The data holder considered the data on polyploidy in the chromosome aberration study with Aclonifen to be within historical control levels for the laboratory.

23. The data holder withdrew from the meeting so that the COM could derive its conclusions.

COM DISCUSSIONS

24. The COM noted peer-reviewed scientific literature which provided examples of diphenyl ether breakage in rats, mice and one bacterial strain (*Sphingomonas wittichii*) and considered it was therefore feasible that metabolism of systemic Aclonifen could result in the formation of free (unconjugated) hydroquinone and phenol, although there were no specific data on this aspect. The COM considered if exogenous metabolic activation systems such as Arochlor-1254 could metabolise Aclonifen to hydroquinone and phenol and agreed there were no specific data available.

25. The COM discussed the revised metabolism pathway for Aclonifen submitted by the data holder and agreed the proposal was feasible but not supported by appropriate data. Members were informed by the data holder that formation of phenol and hydroquinone prior to conjugation was equally unsupported as a second hypothesis in terms of available data. Members noted the proposal from the data holder that, if hydroquinone and phenol were formed from Aclonifen, then some positive results should have been recorded in the mutagenicity studies on Aclonifen.

26. The COM considered that the comparisons made between mutagenicity of Aclonifen and hydroquinone and phenol were useful but had reservations regarding whether definite conclusions could be reached. Thus it was possible that, when Aclonifen was orally administered to mice, hydroquinone and phenol were formed but failed to induce a detectable increase in micronucleus frequency in the polychromatic erythrocytes of the bone marrow.

COM CONCLUSIONS

27. The COM agreed that further data on Aclonifen metabolism was required. This could involve more *in vivo* tests with specific analysis for the formation of hydroquinone and phenol. Alternatively, it might be possible to undertake comparative *in-vitro* studies using rodent and human tissues (with specific measurement of hydroquinone and phenol formation). It was considered this could provide evidence that exposure to Aclonifen was unlikely to be associated with significantly increased genotoxic risk, although this would not preclude the possible need for additional mutagenicity tests dependent on the outcome of the metabolism studies.

28. The COM noted the approach to risk assessment had not been considered during the presentation, but that the data holder had included a proposed Margin of Exposure approach in the submission dated 13 August 2008. This would need to be considered further when appropriate metabolism data were available.

November 2008

REFERENCES

1. COM statement on hydroquinone and phenol. COM/00/S1. January 2000.
2. COM statement on phenol (2008).
<http://www.iaacom.org.uk/statements/StatementsChronological.htm>
3. Fisher P. Position paper on the metabolic fate of Aclonifen in the rat: cleavage of the diphenyl ether bond revisited. 21 October 2008. Bayer CropScience.
4. Extract from detailed record of ACP consideration of Aclonifen. ACP meeting 329.
5. Extracts from draft EU risk assessment report on metabolism and genotoxicology of Aclonifen.
6. Kramer PJ. *In-vitro* assessment for the mutagenic potential in bacteria with and without addition of a metabolising system. Inst of Toxicology, E. Merck, Darmstadt, 08.04.1982, KUB 3358.
7. Herbold B. Salmonella/microsome test. Plate incorporation and preincubation method. Report AT02825. AE F068300 (Aclonifen). Study T1076125. TXCLX014. Bayer Health Care AG, PH-GDD Toxicology, 42096 Wuppertal, Germany.

8. Dance CA. *In-vitro* assessment of the clastogenic activity of Aclonifen in cultured human lymphocytes. LSR Report 92/RHA477/0471. Life Science Research, Eye, Suffolk, England.
9. Anonymous. Mammalian cell (V79) mutagenicity test on Aclonifen. Report SP 579/VT-19, Institute of Toxicology, University of Mainz, Obero Zahlbacher, Straß3, 67, Germany. 6 March 1984.
10. Anonymous. Unscheduled DNA synthesis (UDS) in primary rat hepatocytes (Autoradiographic method). Aclonifen Technical. RTC report 121009-M-03691. Research Toxicology Centre S.p.A. 31/10/91.
11. Anonymous. Cytogenetic Investigations in NMRI Mice after single oral administration of CME 127 (Aclonifen). Micronucleus test. Project 26M0286/8332. Translation Celamerck Document 127AD-457-005. March 8, 1984.
12. Sagelsdorff P. Investigation of the potential for DNA-binding of Aclonifen. CIBA-Geigy Ltd. Toxicology/Cell Biology CH-4002 Basel. Project CB95/24, 15 August 1995.
13. Semino G, Mackenzie E and Leake C. Position paper on the relevance of phenol and hydroquinone formation following Aclonifen exposure. 13 August 2008. Bayer CropScience.
14. Qiu X, Mercado-Feliciano M, Bigsby RM, Hites RA. Measurement of polybrominated diphenyl ethers and metabolites in mouse plasma after exposure to a commercial pentabromodiphenyl ether mixture. *Environ Health Perspect.* 2007 Jul;115(7):1052-8.
15. Keum YS, Lee YJ, Kim JH. Metabolism of nitrodiphenyl ether herbicides by dioxin-degrading bacterium *Sphingomonas wittichii* RW1. *J Agric Food Chem.* 2008 Oct 8;56(19):9146-51.
16. Chen LJ, Lebetkin EH, Sanders JM, Burka LT. Metabolism and disposition of 2,2',4,4',5-pentabromodiphenyl ether (BDE99) following a single or repeated administration to rats or mice. *Xenobiotica.* 2006 Jun;36(6):515-34.
17. Balsam A, Sexton F, Borges M, Ingbar SH. Formation of diiodotyrosine from thyroxine. Ether-link cleavage, an alternate pathway of thyroxine metabolism. *J Clin Invest.* 1983 Oct;72(4):1234-45.