

Evaluating endocrine activity in leaf litter using *in vitro* screens for androgenic, oestrogenic and progestogenic activity.

Authors:

Brian, J.V.¹, Beresford, N.¹, Smith, A.², White, S.², Bean, T.³,
Katsiadaki, I.³, Sebire, M.³ & Hutchinson, T.H.^{3*}

¹Institute for the Environment, Brunel University, Uxbridge, Middlesex UB8 3PH, UK; ²Cefas Lowestoft Laboratory, Pakefield Road, Lowestoft, Suffolk NR33 0HT, UK; & ³Cefas Weymouth Laboratory, The Nothe, Weymouth, Dorset DT4 8UB, UK.

*Corresponding author.

Issue date: January 2012

Executive Summary

1.1 As part of the CEFIC Long-range Research Initiative (LRi) project CEFIC RfP LRI-EMSG55, aqueous and ethanol extracts of fallen leaves from beech (*Fagus sylvatica*) and oak (*Quercus robur*) and standing dead stems of the common reed (*Phragmites australis*) have been screened in the yeast androgen screen (YAS), yeast oestrogen screen (YES) and the yeast progesterone in screen (YPS). The YAS, YES and YPS assays were all run to assess both potential agonist and antagonist activity for leaf samples collected from south-west England in 2011.

1.2 Leaf material (sample code in capitals) was collected from three Devon (UK) sites: BEECH-2011-1 from Staverton Woods (collected 24 April 2011); OAK-2011-1 from Totnes (collected 11 March 2011); and REED-2011-1 from Slapton Ley (collected 6 June 2011). Beech and oak leaves were collected from the surface leaf litter by hand while standing stems and leaves of dead reed were cut by hand. The leaves were then air dried for 7 days, then vacuum packed and stored at -80°C.

1.3 To prepare the aqueous extracts, 450 g of dried leaves from each species were ground to a fine powder and were extracted in synthetic freshwater medium overnight at different temperatures (4°, 15° or 20°), then centrifuged and stored at -80°C until required. The concentration of dissolved organic carbon (DOC) in the extracts was analyzed by liquid chromatography organic carbon detection. Aqueous extracts were concentrated using solid phase extraction and eluted with ethanol forming solvent extracts, which were also tested for endocrine activity in vitro. Typically, 40L of fallen leaves produced 1.5 kg of dried leaf material and this generated 5 to 6L of aqueous extracts. In terms of DOC results, 24 h extractions at 4°, 15° or 20°C for batch BEECH-2011-1 gave 406, 449 and 665 mg/L DOC, respectively. Extracts of batch OAK-2011-1 at 4°, 15° or 20°C gave 365, 730 and 1030 mg/L DOC, respectively. Extracts of batch REED-2011-1 at 4°, 15° or 20°C gave 650, 941 and 984 mg/L DOC, respectively.

1.4 Since the overall aim of the work was primarily to generate data of direct relevance to freshwater ecosystems, it was decided to first screen aqueous extracts of beech and oak leaves and reed stems. Spare samples of the aqueous extracts are stored at -80°C at Cefas Weymouth and the endocrine screening was successfully completed through a Cefas – Brunel University (BU) cooperation. Supplementary work was then undertaken using the (agonist and antagonist) YAS, YES and YPS assays to evaluate endocrine activity of ethanol extracts derived from these aqueous aliquots.

1.5 Based on a single sample of leaves collected for each plant species in 2011, endocrine activity was detected in all aqueous extracts in the anti-YAS, YES and anti-YES assays. The main observations

were that oak (ref OAK-2011-1) and reed (ref REED-2011-1) samples were most active, the latter showing DOC-related responses in YES (agonist) assays run by both Brunel University and Cefas. The oak (ref OAK-2011-1) sample showed both anti-YAS and anti-YES activity.

1.6 Bearing in mind the semi-quantitative nature of the yeast screens and the ethical issues associated with in vivo fish testing of leaf extracts on the basis of preliminary data, further work is planned during 2012 to seek to repeat these findings before moving to in vivo fish studies. If feasible within the time and budget constraints, it would be desirable to screen beech, oak and reed samples in novel in vitro fish transgenic receptor assays currently being developed in a separate on-going Cefas project. Following discussions with the sponsor in November 2011, further samples of oak and reed will be collected in 2012 for assessing endocrine activity at a range of environmentally relevant DOC values (eg between 5 and 50 mg/L DOC).