only if he is willing to count essentially the same sentence several times. To do so, however, serves as a good example of the practice of double-counting which we feel is invited by the proposed standards.

We are concerned about the bureaucratic inclination to measure particular kinds of so-called "benefits," which tend to be but special categories of the general concept of gains in national economic welfare. This inclination leads to double-counting and is especially troublesome if the agency pleads lack of expertise when it comes to measuring costs. As economists, we see great flexibility in the national economic efficiency account to include, albeit only once, all of the types of benefits and costs that are discussed by Major and the Water Resources Council. Also, we are wary when the agency that constructs also performs the evaluation analyses.

To repeat the main thrust of our critique, it seems unwarranted for water resource planners to establish a set of standards emphasizing nonefficiency effects and secondary impacts, when their performance in accurately appraising the relatively easy-to-measure primary efficiency effects has been so inadequate. We say this while at the same time acknowledging the relevance of information on these nonefficiency impacts to project appraisal and choice.

In conclusion, we have two principal concerns. First, a separation of evaluation and construction must precede any accounting change. Second, when well-defined special interests benefit from a construction project, the collection of user costs is absolutely necessary.

CHARLES CICCHETTI
Social Systems Research Institute and
Department of Economics,
University of Wisconsin,
Madison 53706

ROBERT DAVIS
Department of Geography and
Environmental Engineering and
Political Economy, Johns Hopkins
University, Baltimore, Maryland 21218

STEVE HANKE*
Graduate School of Policy Analysis,
University of California,
Berkeley 94720

ROBERT HAVEMAN
Department of Economics,
University of Wisconsin

Notes

* On leave from the Department of Geography and Environmental Engineering and Political Economy, Johns Hopkins University, Baltimore, Maryland 21218.

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Morpholine as Olfactory Stimulus in Fish

Cooper and Hasler (*1*) reported electrophysiological evidence for retention of olfactory cues in homing salmon. They found significant differences in the magnitude of the evoked electroencephalographic (EEG) response to 1 percent morpholine (10^4 mg/liter) for homing coho salmon exposed to morpholine at 5×10^{-5} mg/liter as fingerlings 1 month before smolting as compared to salmon not exposed to morpholine as fingerlings.

Salmon and trout are considered to be macrosmatic on the basis of both behavioral and electrophysiological observations. Recent electrophysiological studies have shown that both salmon and trout respond to certain amino acids at extremely low concentrations, and that stimulatory effectiveness is closely related to molecular structure (*2*). A variety of chemicals such as alcohols, aliphatic acids, and amines, which are highly odorous to humans, are not always stimulatory to these fishes (*2, 3*). There is no evidence, behavioral or electrophysiological, indicating the involvement of olfaction in the detection of morpholine by fish. Wisby (*4*), who first studied the effect of morpholine, found that concentrations as low as 10^{-5} and 10^{-6} mg/liter were repellent to coho salmon fry. However, he failed to show that the salmon detected morpholine by olfaction. The concentrations of morpholine used by Cooper and Hasler (*1*) are well within the range in which morpholine is repellent to salmon fry, whether by olfaction or not. The data reported here suggest that the morpholine effect on which their research is based may be a nonspecific irritational effect elicited by a physiological mechanism other than olfaction.

Rainbow trout (*Salmo gairdneri*) were studied by methods described (*2, 5*). The effect of morpholine at lower concentrations (0.01 and 0.1 percent) is primarily inhibition of the spontaneous background activity followed by a slight afterresponse. At 1 percent, which is the same concentration employed by Cooper and Hasler and approximately 10^8 times higher than that of the lowest threshold determined electrophysiologically for the most stimulatory amino acids, the background EEG activity is slowly replaced by an oscillatory potential that is not terminated by rinsing. Figure 1A shows a typical effect induced by application of 1 percent morpholine in the nares for 10 seconds. The response shown here is for the last of three consecutive stimuli. This morpholine effect differs from the normal olfactory response (for example, Fig. 1B) in that (i) there is a long delay in the morpholine reaction, (ii) the effect builds up gradually and is sustained over a long period after rinsing, and (iii) this period increases with repeated stimulation. This evidence suggests that the morpholine solution may penetrate deep into the olfactory epithelium and cause a nonspecific irritational effect at nonspecialized cell surfaces. It also seems likely that the effect is caused by the high *p*H of the 1 percent morpholine solution (*p*H of 10.2). The normal olfactory response in fish is highly *p*H-dependent and is almost entirely inhibited at *p*H higher than 9 (*6*).

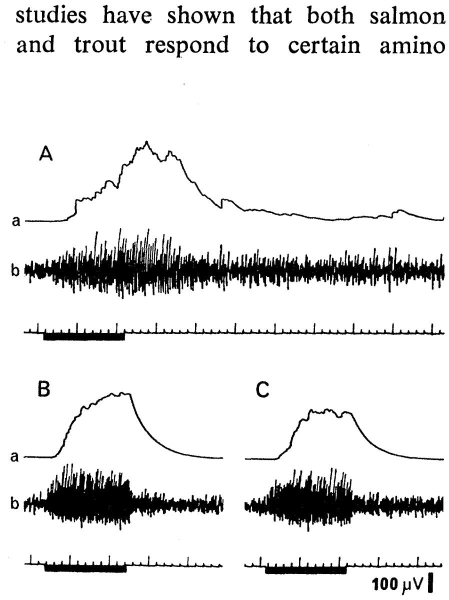

Fig. 1. (A) Effect of 1 percent morpholine on EEG activity of the olfactory bulb of rainbow trout. This record is for the last of three consecutive stimuli, each of 10-second duration and applied at 2-minute intervals. In this record and those in (B) and (C), the upper traces (a) show the integrations of the lower (b). Heavy lines below each record indicate the duration of the stimuli; small hatch marks, 1 second. (B) Typical response to $10^{-5}M$ L-serine. (C) Response to $10^{-5}M$ L-serine after three consecutive applications of 1 percent morpholine (10-second duration, at 2-minute intervals), followed by rinsing for 5 minutes. The response magnitude is reduced compared to (B).

Another serious problem is that the normal olfactory response is markedly inhibited after application of 1 percent morpholine solution into the nares. Figure 1, B and C, illustrates, respectively, the responses to $10^{-5}M$ L-serine before and after three applications of 1 percent morpholine. The response in Fig. 1C, recorded after a 5-minute rinsing, still remains suppressed, with magnitude approximately 75 percent of that of the control response (Fig. 1B).

From these results we conclude that the morpholine effect is probably caused by a mechanism not directly associated with normal olfactory function, and that the 1 percent morpholine solution temporarily inhibits the olfactory sensitivity of rainbow trout.

TOSHIAKI J. HARA
S. MACDONALD

Department of the Environment, Freshwater Institute, Winnipeg, Manitoba R3T 2N6, Canada

References

1. J. C. Cooper and A. D. Hasler, *Science* **183**, 336 (1974).
2. T. J. Hara, *Comp. Biochem. Physiol. A Comp. Physiol.* **44**, 407 (1973).
3. A. M. Sutterlin and N. Sutterlin, *J. Fish. Res. Board Can.* **28**, 565 (1971); N. Suzuki and D. Tucker, *Comp. Biochem. Physiol. A Comp. Physiol.* **40**, 399 (1971).
4. W. J. Wisby, thesis, University of Wisconsin, Madison (1952).
5. T. J. Hara, Y. M. C. Law, E. van der Veen, *J. Fish. Res. Board Can.* **30**, 283 (1973).
6. T. J. Hara, in preparation.

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Hara and Macdonald are correct in stating that we assume that morpholine is detected by olfaction. Morpholine is detected at very low concentrations (*1*, *2*). Since no other sensory system is known to operate at such low concentrations, olfaction is considered to be the sensory modality. Recent research on taste indicates that this chemoreceptor system might also be considered (*3*), although highly developed taste receptors are not known for salmonids.

It would appear that fish show specific homing behavior only if the odor or odors have biological significance to them. Otherwise, a novel odor may elicit avoidance or alarm responses or no apparent response at all. Wisby (*1*) found that many organic chemicals were aversive to naive fish. Since fish exposed to morpholine as juveniles returned to a stream scented with morpholine at repellent concentrations (*2*, *4*), imprinted fish were different from naive fish, that is, controls.

Even if one assumes that 1 percent morpholine solutions act as a general irritant, Hara and Macdonald have not explained why our control and imprinted groups of fish showed significantly different responses to this chemical. We have reproduced these results in studies of three groups of imprinted coho salmon and their controls (*5*, *6*). Similar results were obtained with imprinted rainbow trout (*5*, *7*). Thus, the responses elicited by 1 percent morpholine are certainly not nonspecific, as would be expected if morpholine acted as a general irritant. Moreover, the electroencephalographic (EEG) work is supported by ultrasonic tracking and census studies on homing coho salmon and rainbow trout (*4*, *5*).

We acknowledge that 1 percent morpholine, the concentration used in the EEG technique, is extremely high when compared with the imprinting concentration of 5×10^{-5} mg/liter. This problem of threshold, however, is not unique. As we reported (*8*), Hara (*9*) found that the EEG threshold for L-serine is 10^{3} times higher than that observed in behavioral experiments. We detected significant differences in evoked potentials in response to stream water scented with morpholine at about 10^{-3} mg/liter, a concentration 10^{3} times higher than that in behavioral experiments (*4*, *5*). Therefore, responses to morpholine show the same difference in threshold as do responses to amino acids and other chemicals tested by Hara.

Thus, responses to morpholine are seen at very high and very low concentrations; although there are no responses at intermediate concentrations, such a difference in sensitivity is known for another environmental factor, magnetism. While high magnetic fields (100 to 1000 times greater than that of the earth's) produce some behavioral effects, fields that are only 10 to 30 percent higher than that of the earth's can, for instance, significantly disorient bees performing the waggle dance (*10*).

Hara and Macdonald suggest that *p*H may be responsible for the evoked potentials produced by 1 percent morpholine. We discussed this possibility (*8*). If responses to 1 percent morpholine were due to *p*H only, then one might expect no differences in responses between imprinted and control fish. Buffer solutions of *p*H 9.5 did not elicit significant differences in response in these groups. Therefore, responses were probably caused by something other than *p*H.

We have referred to a priming effect of stimulatory chemicals, that is, a buildup in response as the sample presentation is repeated (*11*). The experiments reported by Hara and Macdonald are not directly comparable, because different methods were used. We found no evidence that the amplitudes of responses to L-methionine were significantly affected by prior exposure to 1 percent morpholine. These results apply to rainbow trout as well.

In summary, ablation studies are needed to resolve whether morpholine is detected by olfaction. However, there is ample behavioral evidence that olfaction is important in this system.

JON C. COOPER
ARTHUR D. HASLER

Laboratory of Limnology, University of Wisconsin, Madison 53706

References

1. W. J. Wisby, thesis, University of Wisconsin, Madison (1952).
2. A. D. Hasler and W. J. Wisby, *Am. Nat.* **85**, 223 (1951); D. M. Madison, A. T. Scholz, J. C. Cooper, R. M. Horrall, A. D. Hasler, A. E. Dizon, *Fish. Res. Board Can. Tech. Rep. No. 414* (1973); A. T. Scholz, J. C. Cooper, D. M. Madison, R. M. Horrall, A. D. Hasler, A. E. Dizon, R. Poff, in *Proceedings of the 16th Conference of International Association for Great Lakes Research*, Sandusky, Ohio, April 1973 (Braun-Brumfield, Ann Arbor, Mich., 1973), pp. 143–153.
3. D. Tucker, *J. Fish. Res. Board Can.* **30**, 1243 (1973); B. Oakley, personal communication.
4. A. T. Scholz, J. C. Cooper, R. M. Horrall, A. D. Hasler, in preparation.
5. J. C. Cooper, thesis, University of Wisconsin, Madison (1974).
6. ——— and A. D. Hasler, in preparation.
7. J. C. Cooper, A. T. Scholz, A. D. Hasler, in preparation.
8. J. C. Cooper and A. D. Hasler, *Science* **183**, 336 (1974).
9. T. J. Hara, *J. Fish. Res. Board Can.* **29**, 1351 (1972).
10. H. Martin and M. Lindauer, *Fortschr. Zool.* **21**, 211 (1973).
11. J. C. Cooper and A. D. Hasler, *Fish. Res. Board Can. Tech. Rep. No. 415* (1973).

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